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CONTENTS OF VOLUME 29

FEBRUARY, 1943, NUMBER 1.

HENRY E. MELENEY, Portrait	Frontispiece
Meleney, Henry E. THE RÔLE OF PARASITOLOGISTS IN WORLD WAR II	1
Fischthal, Jacob H. A DESCRIPTION OF <i>Phyllodistomum etheostomae</i> FISCHTHAL, 1942 (TREMATODA: GORGODERIDAE), FROM PERCID FISHES	7
Rutherford, Robert L. THE LIFE CYCLE OF FOUR INTESTINAL COCCIDIA OF THE DOMESTIC RABBIT	10
Wheeler, Charles M. A CONTRIBUTION TO THE BIOLOGY OF <i>Ornithodoros hermsi</i> WHEELER, HERMS AND MEYER	33
Whitlock, J. H. CHARACTERISTICS OF THE POPULATION AVAILABLE FOR BIOASSAY OF ANT- HELMINTICS IN <i>Nippostrongylus muris</i> INFECTION IN ALBINO RATS	42
Whitlock, J. H. and C. I. Bliss. A BIOASSAY TECHNIQUE FOR ANTHELMINTICS	48
Brody, Arthur L. and Edward F. Knippling. CAN LARVAE OF <i>Cochliomyia americana</i> C. AND P. MATURE IN CARCASSES?	59
Larsh, John E., Jr. THE RELATIONSHIP BETWEEN INTESTINAL SIZE OF YOUNG MICE AND THEIR SUSCEPTIBILITY TO INFECTION WITH THE CESTODE, <i>Hymenolepis nana</i> var. <i>fra-</i> <i>terna</i>	61
Beaver, Paul C. STUDIES ON <i>Protechinostoma mucronisertulatum</i> , N. G., N. N. (<i>Psilosto-</i> <i>mun reflexae</i> FELDMAN, 1941), A TREMATODE (ECHINOSTOMIDAE) FROM THE SORA RAIL	65
Hunninen, A. V. and R. M. Cable. THE LIFE HISTORY OF <i>Lecithaster confusus</i> ODHNER (TREMATODA: HEMIURIDAE)	71
RESEARCH NOTE.	
Steinhaus, Edward A. A NEW BACTERIUM, <i>Corynebacterium lipoptenae</i> , ASSOCIATED WITH THE LOUSE FLY, <i>Lipoptena depressa</i> SAY	80

APRIL, 1943, NUMBER 2.

Cort, W. W. and Louis Olivier. THE DEVELOPMENT OF THE LARVAL STAGES OF <i>Plagiorchis</i> <i>muris</i> TANABE, 1922, IN THE FIRST INTERMEDIATE HOST	81
Goodnight, Clarence J. REPORT ON A COLLECTION OF BRANCHIOBELLEIDS	100
Lincicome, David Richard. ACANTHOCEPHALA OF THE GENUS <i>Corynosoma</i> FROM THE CALI- FORNIA SEA-LION	102
Hurlbut, Herbert S. THE RATE OF GROWTH OF <i>Anopheles quadrimaculatus</i> IN RELATION TO TEMPERATURE	107
Culbertson, James T. NATURAL TRANSMISSION OF IMMUNITY AGAINST <i>Trichinella spiralis</i> FROM MOTHER RATS TO THEIR OFFSPRING	114
Peters, Harold T. STUDIES ON THE BIOLOGY OF <i>Anopheles walkeri</i> THEOBALD (DIPTERA: CULICIDAE)	117
Fischthal, Jacob H. NUMBER OF LARVAE AND TIME REQUIRED TO PRODUCE ACTIVE IM- MUNITY IN RATS AGAINST <i>Trichinella spiralis</i>	123
Van Cleave, Harley J. and Charles O. Williams. MAINTENANCE OF A TREMATODE, <i>As-</i> <i>pidogaster conchicola</i> , OUTSIDE THE BODY OF ITS NATURAL HOST	127
Bequaert, J. NOTES ON HIPPOBOSCIDAE. 16. HIPPOBOSCIDAE FROM SOUTHERN BRASIL. WITH THE DESCRIPTION OF A NEW SPECIES OF <i>Lynchia</i>	131
Ferguson, M. S. DEVELOPMENT OF EYE FLUKES OF FISHES IN THE LENSES OF FROGS, TUR- TLES, BIRDS, AND MAMMALS	136
Stunkard, Horace W. A NEW TREMATODE, <i>Dictyanguium chelydrae</i> (MICROSCAPHIDIIDAE = ANGIODICTYIDAE), FROM THE SNAPPING TURTLE, <i>Chelydra serpentina</i>	143
Jones, William R. and Howard A. Jones. PRELIMINARY OBSERVATIONS ON THE EFFICACY OF A PRODUCT FROM OIL OF ROSE GERANIUM FOR THE REMOVAL OF INTESTINAL PARASITES FROM DOGS	151
RESEARCH NOTES.	
Thompson, Paul E. THE RELATIVE INCIDENCE OF BLOOD PARASITES IN SOME BIRDS FROM GEORGIA	153
Annereaux, R. F. <i>Opecoelina pharynmagna</i> N. SP. (TREMATODA) FROM THE CHINA ROCKFISH	155
Chandler, Asa C. A REDESCRIPTION OF <i>Contracaecum habena</i> (LINTON, 1900) LIN- TON, 1934	156

Chandler, Asa C. A CASE OF CANINE STRONGYLOIDIASIS IN TEXAS	157
Goble, Frans C. NOTES ON THE ADULTS OF <i>Protostrongylus coburni</i> IN THE LUNGS OF WHITE-TAILED DEER	158
Morgan, Banner Bill. NEW HOST RECORDS OF NEMATODES FROM MUSTELIDAE (CARNIVORA)	158
Graham, Edward and Jacob Uhrich. ANIMAL PARASITES OF THE FOX SQUIRREL, <i>Sciurus niger rufiventer</i> , IN SOUTHEASTERN KANSAS	159
Rogers, William P. <i>Strongyloides planiceps</i> , NEW NAME FOR <i>S. cati</i> ROGERS	160
COMMUNICATION.	
Hunter, George W., III. SPECIMENS NEEDED BY THE MEDICAL SCHOOLS	160

JUNE, 1943, NUMBER 3.

Webster, J. Dan. HELMINTHS FROM THE ROBIN, WITH THE DESCRIPTION OF A NEW NEMA- TODE, <i>Porrocaecum brevispiculum</i>	161
Cort, W. W. and Louis Olivier. THE DEVELOPMENT OF THE SPOROCYSTS OF A SCHISTO- SOME, <i>Cercaria stagnicolae</i> TALBOT, 1936	164
Pratt, Ivan and William D. Lindquist. THE MODIFICATION OF THE DIGESTIVE GLAND TUBULES IN THE SNAIL <i>Stagnicola</i> FOLLOWING PARASITIZATION	176
Hedrick, Leslie R. TWO NEW LARGE-TAILED CERCARIAE (PSILOSTOMIDAE) FROM NORTH- ERN MICHIGAN	182
Wood, Sherwin F. and Carlton M. Herman. THE OCCURRENCE OF BLOOD PARASITES IN BIRDS FROM SOUTHWESTERN UNITED STATES	187
Wallace, F. G. FLAGELLATE PARASITES OF MOSQUITOES WITH SPECIAL REFERENCE TO <i>Crithidia fasciculata</i> LÉGER, 1902	196
Herman, Carlton M., John E. Chattin and Roy W. Saarni. FOOD HABITS AND INTENSITY OF COCCIDIAN INFECTION IN NATIVE VALLEY QUAIL IN CALIFORNIA	206
Russell, Paul F., Badri Nath Mohan and Persis Putnam. SOME OBSERVATIONS ON SPLEEN VOLUME IN DOMESTIC FOWLS IN THE COURSE OF <i>Plasmodium gallinaceum</i> STUDIES	208
Byrd, Elon E. and James W. Ward. OBSERVATIONS ON THE SEGMENTAL ANATOMY OF THE TAPEWORM, <i>Mesocestoides variabilis</i> MUELLER, 1928, FROM THE OPOSSUM	217
Meserve, F. G. <i>Phyllodistomum coatneyi</i> N. SP., A TREMATODE FROM THE URINARY BLADDER OF <i>Ambystoma maculatum</i> (SHAW)	226
RESEARCH NOTES.	
Beaver, Paul C. A TRAY FOR COLLECTING ANOPHELINE MOSQUITO LARVAE	229
Reid, W. M. A PHYSALOPTERAN (NEMATODA) FROM THE DOMESTIC PIG	229
Webster, J. Dan. THE TYPE OF <i>Gyrocœlia milligani</i> LINTON, 1927	230
Goble, Frans C. and E. L. Cheatum. <i>Dispharynx spiralis</i> IN GOLDEN AND RING- NECKED PHEASANTS IN NEW YORK	230
Luttermoser, George W. A NOTE ON THE LIFE CYCLE OF <i>Australorbis glabratus</i> (SAY, 1818) PILSBRY, 1934, A SNAIL INTERMEDIATE HOST OF <i>Schistosoma mansoni</i>	231
IN MEMORIAM. ALBERT HASSALL (1862-1942)	232
AMERICAN SOCIETY OF PARASITOLOGISTS. MINUTES OF 31ST COUNCIL MEETING, NEW YORK CITY, JANUARY 9, 1943	236

AUGUST, 1943, NUMBER 4.

Highby, Paul R. <i>Dipetalonema arbuta</i> N. SP. (NEMATODA) FROM THE PORCUPINE, <i>Erethizon</i> <i>dorsatum</i> (L.)	239
Highby, Paul R. MOSQUITO VECTORS AND LARVAL DEVELOPMENT OF <i>Dipetalonema arbuta</i> HIGHBY (NEMATODA) FROM THE PORCUPINE, <i>Erethizon dorsatum</i>	243
Highby, Paul R. VECTORS, TRANSMISSION, DEVELOPMENT, AND INCIDENCE OF <i>Dirofilaria</i> <i>scapiceps</i> (LEIDY, 1886) (NEMATODA) FROM THE SNOWSHOE HARE IN MINNESOTA	253
Jordan, Helen B. BLOOD PROTOZOA OF BIRDS TRAPPED AT ATHENS, GEORGIA	260
Sarles, Merritt P. OVERWINTER LOSS OF NODULAR WORM LARVAE FROM A SHEEP PASTURE AND ITS BEARING ON THE CONTROL OF NODULAR WORM DISEASE	263
Byrd, Elon E. and James W. Ward. NOTES ON THE GENITAL SYSTEM OF THE BIRD FLUKE, <i>Apharyngostrigea cormi</i> (ZEDER)	270
Packchanian, A. ON THE VIABILITY OF VARIOUS SPECIES OF <i>Trypanosoma</i> AND <i>Leishmania</i> CULTURES	275
Snyder, Thomas L. and Henry E. Meleney. ANAEROBIOSIS AND CHOLESTEROL AS GROWTH REQUIREMENTS OF <i>Endamoeba histolytica</i>	278

Shorb, D. A. SURVIVAL ON GRASS PLOTS OF EGGS AND PREINFECTIONAL LARVAE OF THE COMMON SHEEP STOMACH WORM, <i>Haemonchus contortus</i>	284
Ward, Helen L. A REDESCRIPTION OF <i>Polymorphus obtusus</i> VAN CLEAVE, 1918 (ACANTHOCEPHALA)	289
Nelson, E. Clifford. CULTIVATION OF <i>Nyctotherus cordiformis</i>	292
RESEARCH NOTES.	
Donaldson, Alan W. THE PREVALENCE OF PINWORM INFECTION IN AN OHIO INSTITUTION FOR CHILDREN	298
Dence, Wilford A. A LEECH FEEDING ON LIGULA	299
Hawkins, Philip A. <i>Sarcocystis rileyi</i> (STILES, 1893) IN THE DOMESTIC FOWL, <i>Gallus gallus</i>	300
Jachowski, Leo, Jr. THE ORIENTAL RAT FLEA (<i>Xenopsylla cheopis</i>) IN MICHIGAN ..	300
IN MEMORIAM. WILLIAM ALBERT HOFFMAN (1894-1943)	301

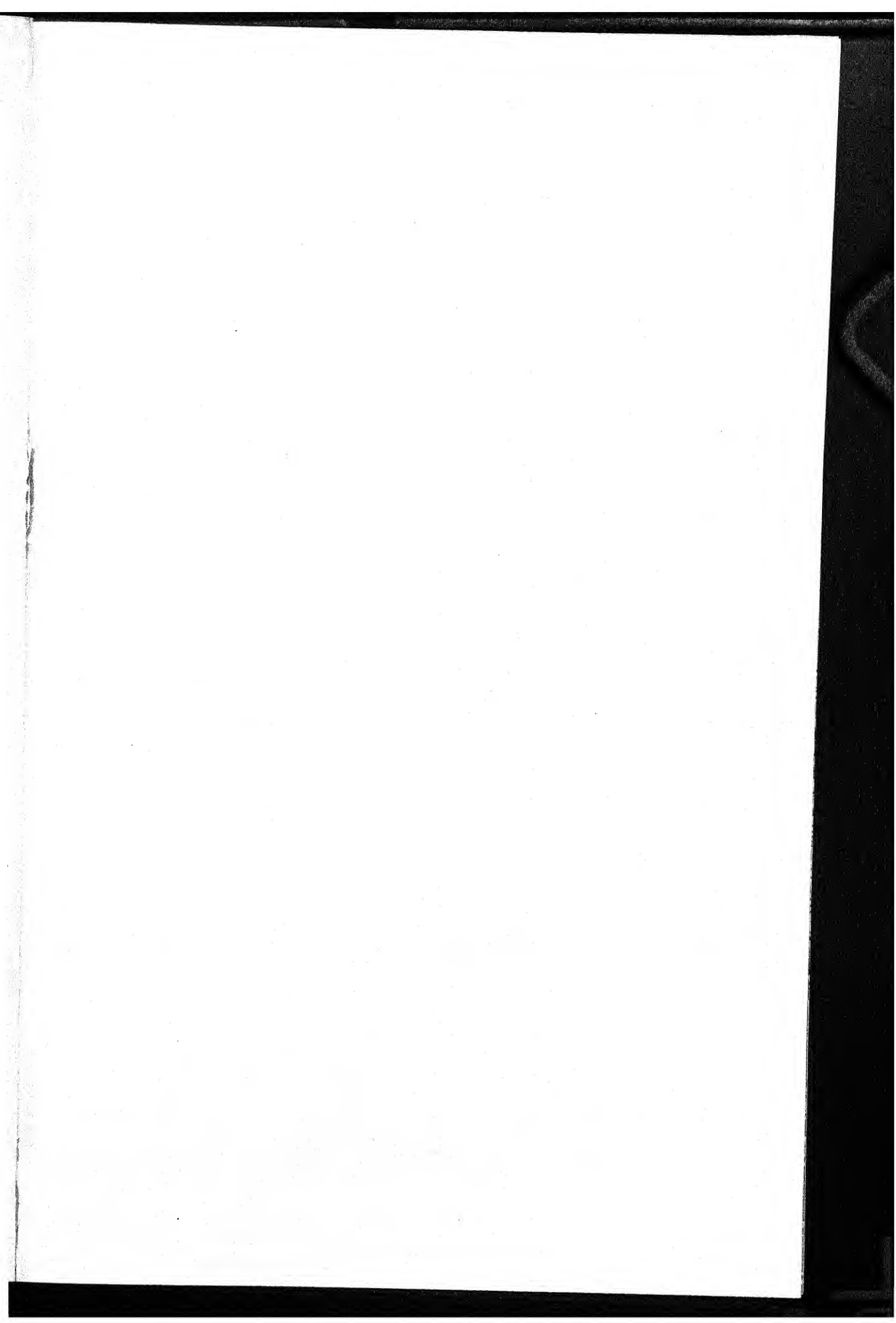
OCTOBER, 1943, NUMBER 5.

Spurlock, G. M. OBSERVATIONS ON HOST-PARASITE RELATIONS BETWEEN LABORATORY MICE AND <i>Nematospiroides dubius</i> BAYLIS	303
Dalmat, Herbert T. A CONTRIBUTION TO THE KNOWLEDGE OF THE RODENT WARBLE FLIES (CUTEREBRIDAE)	311
Ferguson, M. S. IN VITRO CULTIVATION OF TREMATODE METACERCARIAE FREE FROM MICROORGANISMS	319
Thomas, H. Duke. PRELIMINARY STUDIES ON THE PHYSIOLOGY OF <i>Aedes aegypti</i> (DIPTERA: CULICIDAE). I. THE HATCHING OF THE EGGS UNDER STERILE CONDITIONS	324
Philip, Cornelius B. FLOWERS AS A SUGGESTED SOURCE OF MOSQUITOES DURING ENCEPHALITIS STUDIES, AND INCIDENTAL MOSQUITO RECORDS IN THE DAKOTAS IN 1941	328
Brooks, F. G. LARVAL TREMATODES OF NORTHWEST IOWA. I. NINE NEW XIPHIDIOCERCARIAE	330
Brooks, F. G. LARVAL TREMATODES OF NORTHWEST IOWA. II. FOUR NEW STRIGEIDS ...	340
Brooks, F. G. LARVAL TREMATODES OF NORTHWEST IOWA. III. A NEW COLLARLESS ECHINOSTOME CERCARIA	347
Ferguson, M. S. EXPERIMENTAL STUDIES ON THE FISH HOSTS OF <i>Posthodiplostomum</i> (TREMATODA: STRIGEIDA)	350
DeVult, H. M. A NEW MEDIUM FOR THE CULTIVATION OF <i>Histomonas meleagridis</i>	353
Hurlbut, Herbert S. OBSERVATIONS ON THE USE OF SEA WATER IN THE CONTROL OF <i>Anopheles albimanus</i> WIED	356
RESEARCH NOTES.	
Sealand, John A. NOTES ON SOME PARASITES OF THE MINK IN SOUTHERN MICHIGAN	361
Schechter, Victor. TWO FLATWORMS FROM THE OYSTER-DRILLING SNAIL, <i>Thais floridana haysae</i> CLENCH	362
Levine, P. P. THE SELECTIVE ACTION OF SULFAGUANIDINE ON AVIAN COCCIDIA	362
Wood, Sherwin F. A NEW LOCALITY FOR <i>Trypanosoma vespertilionis</i> (= <i>T. cruzi</i> ?) IN BATS IN THE UNITED STATES	363
IN MEMORIAM. WINFIELD CAREY SWEET (1891-1942)	364
AMERICAN SOCIETY OF PARASITOLOGISTS. NOTICE OF POSTPONEMENT OF ANNUAL MEETING OF THE SOCIETY	366

DECEMBER, 1943, NUMBER 6.

Hamann, C. B. ESTIMATION OF HISTAMINE IN THE BLOOD AND OTHER TISSUES OF RATS AND GUINEA PIGS INFECTED WITH <i>Trichinella spiralis</i>	367
Jones, Arthur W. A FURTHER DESCRIPTION OF <i>Stempellia moniezi</i> JONES, 1942, A MICROSPORIDIAN PARASITE (NOSEMATIDAE) OF CESTODES	373
Crawford, Wiley W. COLORADO TREMATODE STUDIES. I. A FURTHER CONTRIBUTION TO THE LIFE HISTORY OF <i>Crepidostomum farionis</i> (MÜLLER)	379
Davis, H. S. A NEW POLYMASTIGINE FLAGELLATE, <i>Costia pyriformis</i> , PARASITIC ON TROUT	385
Manter, Harold W. ONE SPECIES OF TREMATODE, <i>Neorenifer grandispinus</i> (CABALLERO, 1938), ATTACKED BY ANOTHER, <i>Mesocercaria mercianae</i> (LARUE, 1917)	387
Davis, Gordon E. STUDIES ON THE BIOLOGY OF THE ARGASID TICK, <i>Ornithodoros nicollei</i> MOOSER	393
Davis, Gordon E. FURTHER ATTEMPTS TO TRANSMIT <i>Pasteurella tularensis</i> BY THE BED-BUG (<i>Cimex lectularius</i>)	395
Goble, Frans C. and Ellsworth C. Dougherty. NOTES ON THE LUNGWORMS (GENUS <i>Protostrongylus</i>) OF VARYING HARES (<i>Lepus americanus</i>) IN EASTERN NORTH AMERICA	397

Todd, A. C. <i>Thelastoma icemi</i> (SCHWENK), A NEMATODE OF COCKROACHES	404
Stoll, Norman R. THE WANDERING OF <i>Haemonchus</i> IN THE SHEEP HOST	407
Larsh, John E., Jr. INCREASED INFECTIVITY OF THE EGGS OF THE DWARF TAPEWORM (<i>Hymenolepis nana</i> var. <i>fraterna</i>) FOLLOWING STORAGE IN HOST FECES	417
Vail, Edward L. and G. F. Augustson. A NEW ECTOPARASITE (ACARINA: CHEYLETIDAE) FROM DOMESTIC RABBITS	419
RESEARCH NOTES.	
Kirby, Harold. OBSERVATIONS ON A TRICHOMONAD FROM THE INTESTINE OF MAN ..	422
Herman, Carlton M. <i>Giardia</i> IN THE BLOOD OF A KANGAROO RAT	423
Larsh, John E., Jr. COMPARING THE PERCENTAGE DEVELOPMENT OF THE DWARF TAPE- WORM, <i>Hymenolepis nana</i> var. <i>fraterna</i> , OBTAINED FROM MICE OF TWO DIFFERENT LOCALITIES	423
Moldonado, José F. A NOTE ON THE LIFE CYCLE OF <i>Tamerlanca bragai</i> (TREMA- TODA: EUCOTYLIDAE)	424
Kuntz, Robert E. CYSTICERCUS OF <i>Taenia taeniaformis</i> WITH TWO STROBILAE	424
Chandler, Asa C. ADDITIONAL RECORDS OF HUMAN INTESTINAL MYIASIS CAUSED BY <i>Eristalis</i>	425
BOOKS AND MONOGRAPHS RECEIVED	426
AMERICAN SOCIETY OF PARASITOLOGISTS.	
OFFICERS	429
CONSTITUTION	431
NOTICE OF ELECTION OF OFFICERS	434
INDEX FOR VOLUME 29, NUMBERS 1-6	435
JOURNAL OF PARASITOLOGY.	
NEW EDITORIAL COMMITTEE	444





Henry E. Melaney

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Number 1

THE RÔLE OF PARASITOLOGISTS IN WORLD WAR II*

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It is with a sense of real regret that this address is presented to the Society in print rather than by word of mouth. The New York members of the Society had looked forward with keen interest to welcoming a large attendance at what should have been one of its most noteworthy meetings. Your courteous gesture in electing to the presidency last year an amateur parasitologist from the medical profession has been accepted with appreciation of the fact that the Society recognizes, particularly in this time of national emergency, the importance of the animal parasites of man and the rôle they may play in winning or losing the world struggle in which we are involved.

The chief interest of the large majority of the parasitologists of the United States has been in the parasites of lower animals rather than in those of man. There are several reasons for this. The field is larger, the parasites are easier to obtain, they can be studied more easily in biological laboratories and do not require association with medical institutions. Furthermore, the animal parasites of man are less prevalent in most parts of the United States than they are in tropical countries. Our nation has not had extensive colonial possessions in the tropics and there has been less interest in intensive study and control of many of the human parasitic diseases than has existed in countries having extensive possessions or aspirations in the tropics.

Ever since the beginning of the present war in Europe in 1939, the Medical Departments of the Armed Forces of the United States have been concerned with the tropical and parasitic diseases which would face our troops if we were forced into the conflict. The point of view of those of us who had been closely associated with plans for the protection of our troops was limited by the focusing of attention on Europe, Africa and Latin America. With the Japanese attack on Pearl Harbor, however, the point of view of the whole nation was changed overnight and one dramatic element in that change was the sudden interest which the professional world and even the laity manifested in tropical diseases. Our horizon was suddenly extended to encompass the entire world and the diseases which would be encountered in the most remote and unprotected regions.

The use of total manpower means the use of each individual in the position where he can be best fitted, and for which he is best prepared, with the adaptation of his talents and experience to the winning of the war. The parasitologist has a place in the picture, and it is my purpose to sketch the picture so as to suggest the rôle that each may be able to play.

* Address of the Retiring President, American Society of Parasitologists, 1942.

Ever since the Spanish-American War the Medical Corps of the Army and Navy have been concerned with studying diseases which occurred among the personnel of those forces in tropical areas, and many of the studies arising from this interest have formed important contributions to the literature of human parasitology. The Veterinary Corps of the Army has also made valuable contributions to the study of diseases among animals used by land forces. The United States Public Health Service has steadily become more active in the study of diseases caused by animal parasites or transmitted by arthropod vectors. The United States Department of Agriculture, through a number of its divisions, has contributed in a most important manner to the health of domestic animals, and to the protection of man from parasites transmitted from lower animals. In addition to these federal agencies, state universities and private institutions have made important contributions to the control of animal parasites. All of these activities have built up a fund of knowledge and a corps of trained personnel which can act as a basis for the effective control of parasitic diseases among men and animals during the present conflict.

When the Surgeon General of the Army requested the National Research Council in the spring of 1940 to establish advisory committees to the Armed Forces in various fields of medicine, one of the first groups formed was the Subcommittee on Tropical Diseases. This Subcommittee of the Committee on Medicine accepted as its first responsibility the preparation of a series of directives for the diagnosis, treatment and prevention of certain tropical diseases. These were first issued as Circular Letter No. 56 of the Surgeon General's Office in June, 1941 (1) and were published as a section of the War Department Technical Manual 8-210 "Guides to Therapy for Medical Officers" in March, 1942. They were revised and a number of new ones added during the summer of 1942, and will be reissued in the near future. The Naval Medical School used these directives as the basis for a bulletin entitled "Notes on Tropical and Exotic Diseases of Naval Importance," the second edition of which was published in November, 1942.

The second activity of the Subcommittee on Tropical Diseases was the encouragement and supervision of research requested by the Armed Forces. Funds provided by the federal government made it possible to support such research through the Office of Scientific Research and Development, and numerous studies have been in progress during the past year or more on malaria, amoebiasis, insecticides and insect repellents, as well as on other tropical diseases outside the field of parasitology. Four members of the Society have been members of the Subcommittee on Tropical Diseases and many more have participated in the research sponsored by the Subcommittee.

The third interest of the Subcommittee on Tropical Diseases has been the encouragement of instruction in tropical diseases to medical officers and medical students. The Army and Naval Medical Schools had always stressed parasitology and tropical medicine in their post-graduate courses for medical officers, but these courses have recently been tremendously strengthened and expanded. In the summer of 1941, on the recommendation of the Subcommittee, the Army Medical School established a special two-months course for medical officers, with a faculty composed of authorities both within and outside of the government services. At first this course could accommodate only thirty students and the assignment of even that number was difficult to obtain. Recently, however, the Secretary of War issued an

order that every unit of troops going to the tropics should be supplied with a medical officer trained in Tropical Medicine. This has led to the expansion of the course to accommodate two hundred students, and in addition the Department of Tropical Medicine at Tulane University has been requested to give a similar course every two months to an additional thirty medical officers.

Other forms of specialized training which the Armed Forces are providing in the field of tropical medicine and parasitology include a short period of field instruction in malaria at the Tennessee Valley Authority and courses in clinical microscopy for medical officers and medical technicians at the army and naval medical schools and at a number of universities throughout the country. Members of the American Society of Parasitologists are participating in practically all of these courses of instruction to personnel in the Armed Forces.

Tropical medicine and parasitology have never held prominent place in the curriculum of most medical schools in the United States. The deplorable lack of instruction in these fields has long been recognized by a few individuals. At the meeting of the Association of American Medical Colleges in October 1941 a committee was appointed to make a study of the teaching of tropical medicine in medical schools. This committee published its preliminary report in the *Journal of the Association* in March 1942 (2), and presented its final report at the meeting of the Association in October (3). This report showed that only a small minority of the schools were giving adequate instruction in either tropical medicine or parasitology and that many of them lacked instructors qualified to teach these subjects.

Meanwhile, the effect of Pearl Harbor had been felt in the medical schools and a great interest developed in improving instruction in these fields. At the suggestion of Colonel James S. Simmons, Chief of the Division of Preventive Medicine of the Office of the Surgeon General of the U. S. Army, the John and Mary R. Markle Foundation made an appropriation to the National Research Council to develop a program of traveling lecturers on tropical medicine. The Markle Foundation has also made an appropriation to the Association of American Medical Colleges to enable instructors from the United States and Canadian medical schools to attend the intensive courses in tropical medicine at the Army Medical School and Tulane University. Each medical school was offered the opportunity of sending a clinical instructor to the Army Medical School and a pre-clinical instructor to Tulane. It was necessary to limit the enrollment in each of these courses to thirty students for the courses beginning January 4th, 1943. Both courses have been fully enrolled and a number of applicants had to be deferred. It is hoped that they can be given an opportunity to attend these courses at a later date. A number of members of the American Society of Parasitologists are included among the attendants at these courses.

Intramural courses in tropical medicine or parasitology anywhere in this country leave much to be desired in providing practical experience with diseases as they occur in the tropics. The armed forces have recognized this but their medical officers will have to get their practical experience in their assignments in the tropics. There is a probability, however, that instructors from medical schools who have taken the intensive courses in tropical medicine described above, may have an opportunity for a brief period of practical experience in the American tropics during the coming year. Colonel Simmons of the Army Medical Corps and General George C. Dunham,

Director of the Division of Health and Sanitation of the Office of the Coordinator of Inter-American Affairs, are cooperating in the development of such opportunities and Mr. Archie S. Woods of the Markle Foundation has expressed active interest in it.

Several medical schools have already strengthened their teaching staffs by the addition of a parasitologist. Some of these have been transferred from academic schools in the same institution while others have been obtained elsewhere. The loss of medical personnel from the staffs of medical schools may make funds available for the employment of a qualified parasitologist. Some medical school authorities still need stimulation to an appreciation of the importance of parasitic diseases in the medical school curriculum. Other schools may require additional funds for an instructor in this field. Parasitologists who are connected with such institutions may be able to assist in solving some of the problems of personnel.

One other aid to the teaching of tropical and parasitic diseases which has received attention is the provision of adequate teaching material. The Army Medical School has established a Distributing Center for parasitological specimens and other material which may be of value. The needs of the medical schools for teaching specimens were ascertained several months ago. The Army Medical School has circulated directions for obtaining material locally, and much material which is difficult to obtain locally will be furnished on request. The Office of the Surgeon General of the Army also plans to make available to medical schools the data which it has been accumulating on the distribution of diseases in tropical countries. Maps showing the world distribution of the important tropical diseases are also being prepared for distribution. The American Medical Association is cooperating in the editing of this material and the Markle Foundation is assisting financially through the National Research Council. This tremendous surge of interest and activity in preparing for the protection of our troops during the present war emphasizes the important rôle which parasitologists are taking and will take in the program from the educational and research points of view.

The opportunities for parasitologists to enter military service in their professional capacities depends mainly upon their individual qualifications. Only the largest military hospitals and laboratories require for routine operation officers who have qualifications limited exclusively to parasitology. Smaller laboratories are under the supervision of medical officers, most of whom have had special training in parasitology; and most of the enlisted personnel have also had a certain amount of training in the diagnosis of parasitic infections. The Army Sanitary Corps provides for non-medical commissioned officers, but bacteriologists are preferred to parasitologists because the routine work of laboratories lies more along bacteriological lines. An individual who has qualifications in both fields is likely to be especially favored in applying for a commission. The Navy has Hospital Volunteer Specialist Commissions in its Hospital Corps somewhat comparable to the Sanitary Corps of the Army.

There are certain assignments for teaching, research or control activities in the Army and Navy, for which experience in certain fields of parasitology are required. There may be greater opportunity for such assignments as the war goes on. Where special investigations have to be carried out or special control measures instituted, personnel with expert training will be required. Malaria is one of these fields, and although malaria units would require medical malariologists, sanitary engineers and

entomologists, there might be a need for protozoologists among the commissioned or enlisted personnel. The same might be true in other fields where protozoan or helminthic diseases become important enough to require special investigations or control measures.

At the request of the Office of the Surgeon General of the Army, Dr. Norman R. Stoll prepared a list of parasitologists who were known to be already in military or other government service, of others who were teaching in medical schools, and of still others who were available and apparently qualified for military service. More recently Dr. Stoll has supplemented this list by one which gives more details as to the experience of some of these persons in human parasitology. These lists should be of great assistance in the selection of parasitologists for military service and there is every indication that opportunities for active service will multiply as the war continues.

I have spoken of the rôle of parasitologists in active military service and in teaching during the present war. There is also a wide field open in research, on the control both of human parasitic diseases and of those of animals and plants. Not only is it necessary to keep our military and civilian population in the best of health, but it is necessary to furnish them with the best of animal and vegetable foods at the least possible cost. Domestic animals must be raised with a minimum of loss and with the maximum of food value. Crops must be improved, saved from the hazards of predatory insects, and preserved for safe delivery after storage and shipping to distant regions. In research laboratories, both institutional and commercial, parasitologists will undoubtedly find increasing opportunities to contribute to the war effort.

It is impossible to cover the field of opportunity in parasitological research, but I should like to mention a few of the unsolved problems in human parasitology. There are many unsolved problems in malaria. We do not yet know what becomes of the sporozoite between its introduction into man by the mosquito and the appearance of the first merozoites in the red blood cells. We have no drug which will attack the parasite in this most important stage when malaria might be prevented in the human host. We don't yet know the cause of the relapse in malaria, whether it is due to the smoldering of the asexual cycle in the spleen pulp where drugs will not reach it in sufficient concentration, or whether it is due to an exo-erythrocytic cycle in the reticulo-endothelial cells of the body. We do not know whether drugs attack the malaria parasite itself within the red cells or in the blood plasma, or whether they have an indirect action through cellular metabolism. No one has ever cultivated the malaria plasmodia outside the host.

The prevention of malaria by the control of mosquitoes still offers great opportunities for research. There is much to be learned about the methods of killing mosquito larvae. Mosquito repellents have only recently been studied in a systematic way, and the same is true of the methods employed in the killing of adult mosquitoes by sprays. All the problems of malaria control in hyperendemic areas will require a tremendous amount of study both during and after the present conflict.

Amoebiasis also presents some unsolved problems. We do not know how effectively sewage treatment prevents cysts from surviving in sludge or effluent. There is evidence that the usual chlorination of drinking water will not kill the cysts. The relative importance of different modes of transmission has not been completely

studied. The relationship of nutrition and bacterial infection to the invasion of the wall of the intestine is not sufficiently understood. *Endamoeba histolytica* has never yet been cultivated free from bacteria, and this has limited the possibilities of studying its action on tissues, its antigenic properties, and its growth requirements. In the treatment of amoebiasis there is room for improvement, particularly the discovery of a drug to replace emetine for parenteral therapy in order to avoid the toxic effects of that drug.

Trypanosomiasis also presents unsolved problems. In the African infections more effective drugs are needed particularly against the Rhodesian strain. There would be a great advantage also in having drugs which could be administered by mouth, both for the treatment and prophylaxis of the infection. Satisfactory methods of controlling tsetse flies are also needed, particularly in view of the fact that airplanes might easily carry these vectors to South America or India, and establish both vector and disease in new areas.

Trypanosoma cruzi is also unconquered with respect to treatment, nor do we know its exact limits of distribution and particularly whether endemic cases occur in the United States.

Leishmaniasis presents a number of unsolved problems. Only recently has convincing evidence been presented of its actual transmission to man by *Phlebotomus*, but we still do not know what factors determine the incubation period or whether the disease will develop at all in certain infected individuals. Better drugs are needed in treatment, especially for the cases in the Sudan which are resistant to antimony preparations. Again we need drugs which can be administered by mouth. The occurrence of visceral leishmaniasis in Brazil is not yet fully understood, particularly in its possible relationship to the cutaneous form of the disease. The relationship of canine to human infections and the importance of the dog as a reservoir host needs further study.

In helminthic infections the field of treatment still presents many opportunities particularly in connection with *Trichinella* and the filarial worms. Schistosomiasis is also a field for further research, including the possibility of its becoming endemic in the United States, the prevention of transmission in the extramammalian cycle, and the discovery of better drugs particularly for treatment by mouth instead of by vein.

These are illustrations of the fields of research which are open in human parasitology during the present war. The real problems in all fields are related to the prevention of human and animal diseases and the promotion of vigorous health. We should not be concerned so much with taxonomy and the morphological aspects of parasitology as with the action of harmful parasites and with methods of preventing their depredations. These are more difficult fields than those concerned with the anatomical structure of parasites, but they offer more promise of helpful results.

The second World War is only the beginning of the struggle which is before us. Unless we win the peace, we shall lose the war. We must not think that we can go back to our former fields of limited activity when the conflict is over. Our program now must be a preparation for world leadership in the future. Not only will the parasitologist be more important in this country after the return of troops with infections acquired in all parts of the world, but there will also be many opportunities for parasitologists to participate in the study and control of diseases throughout the

world. Military occupation followed by civilian guidance in improving health and economic welfare will create posts for research and campaigns of prevention. The personnel for much of this work will have to be trained in this country. Medical schools will appreciate the importance of presenting a world point of view to their students. Postgraduate instruction in tropical medicine and public health will be greatly increased. Philanthropic agencies will offer opportunities for the exchange of highly trained personnel between countries. Commercial interests will appreciate the importance of maintaining health among native employees in order to conserve their investments as well as to benefit the populations with whom they work.

This picture which I have tried to draw of the part which members of this Society are playing and will play in the second World War, should be a source of satisfaction as well as encouragement. There is no reason why the talents of each one should not be used to the fullest extent. Service at home in teaching or research offers just as valuable opportunities as service on the firing line. If any one feels that his talents are not being used to the fullest advantage, let him look about and see whether he can broaden his field of interest or fit himself better into the great mosaic of cooperative effort. Out of this war, with its destruction of peacetime complacency, postponement of meetings and disruption of academic curricula, should come a new era of scientific advancement which will be of benefit to all mankind.

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A DESCRIPTION OF *PHYLLODISTOMUM ETHEOSTOMAE*
FISCHTHAL, 1942 (TREMATODA: GORGODERIDAE),
FROM PERCID FISHES*

JACOB H. FISCHTHAL

During the examination of the urinary bladder of a northern greenside darter, *Etheostoma blennioides blennioides* Rafinesque, collected on March 23, 1941, from the Huron River in Ann Arbor, Michigan, two immature and one mature specimens of a trematode belonging to the genus *Phyllodistomum* Braun, 1899, were found by Mr. Robert E. Kuntz of this laboratory. This material was kindly given to the author who determined it to be a new species. During the summer of 1941, the author found two of 19 northern greenside darters from the Saline River in Saline, Michigan, infected with one and two adult worms, respectively. Further investigations revealed that one of 21 blackside darters, *Hadropterus maculatus* (Girard), and one of 23 northern logperch, *Percina caprodes semifasciata* (De Kay), collected from Honey Creek near Ann Arbor, had one adult worm each in their

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* Contribution from the Department of Zoology, University of Michigan.

urinary bladders. The following description is based upon the study of living specimens, and of whole mounts of specimens fixed in hot 10 per cent formalin, stained in Mayer's paracarmine and mounted in clarite X (Nevillite no. 1).

A preliminary description of this species has appeared in an abstract by Fischthal (1942).

Phyllodistomum etheostomae Fischthal, 1942
(Figs. 1-2)

Specific diagnosis: Phyllodistomum. Body small, narrowly spatulate. Lateral margins of posterior portion of body thrown into two pairs of folds or flutes giving a wavy or undulate appearance. Posterior end of body slightly notched, turning ventrad. Cuticula spineless, bearing sensory papillae on suckers and on surfaces and margins of body. Oral sucker subterminal, larger than acetabulum; sucker ratios: lengths of oral sucker to acetabulum as 1:0.85; widths of same organs as 1:0.74. Mouth subterminal, ventral; esophagus weakly muscular, slender; intestinal crura narrow, ending at posterior fourth of body, lined with conspicuous layer of cells.

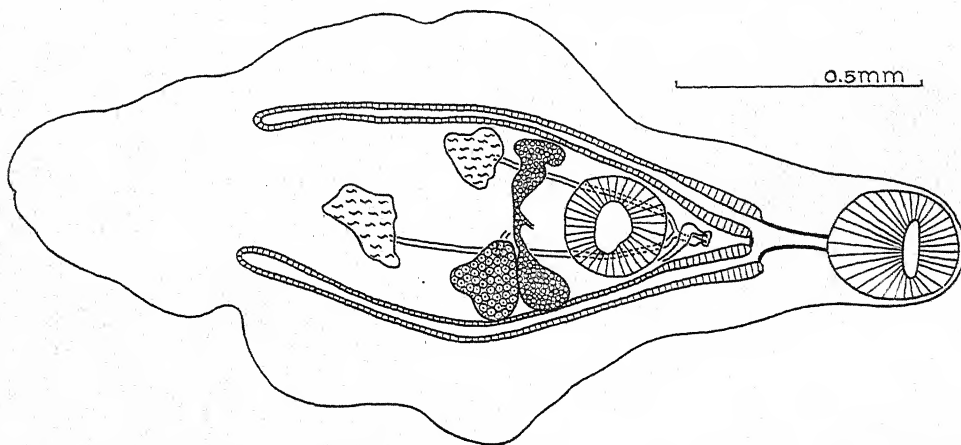


FIG. 1. *Phyllodistomum etheostomae*, adult, ventral view.

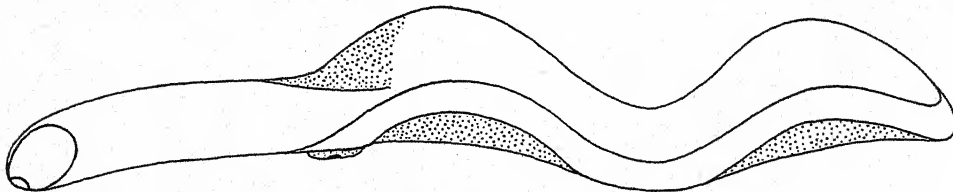


FIG. 2. Lateral view of *Phyllodistomum etheostomae* showing two pairs of marginal folds.

Vitellaria ovoid, smooth or slightly lobate, near and postero-lateral to acetabulum. Vitelline ducts joining dorso-mesially; common vitelline duct short, slightly swollen.

Ovary compact, equal to either testis or slightly larger, slightly and irregularly lobate, usually in contact with vitellarium, amphitypic. Oviduct short, arising from dorsum of ovary. Uterus extensive, coils intra- and extracecal, extending to margins of body posterior to acetabulum; uterus ascending dorsal to acetabulum, constricting to weakly muscular metraterm; genital atrium small. Eggs numerous; older intrauterine eggs containing fully developed miracidia. Measurements of 10 older embryonated intrauterine eggs: $0.031 (0.029-0.032) \times 0.022 (0.021-0.024)$ mm. Testes oblique, well separated, irregularly lobate, indentations deeper and lobes more numerous than in ovary. Anterior testis not in contact with vitellarium and opposite or slightly oblique to ovary. Posterior testis behind but never in contact with ovary. Vasa efferentia arising from dorsum of testes, passing forward dorsal to acetabulum to their junction slightly anterior to acetabulum; vas deferens short; seminal vesicle small; ejaculatory duct short. Genital pore mid-ventral, approximately half-way between intestinal bifurcation and acetabulum. Excretory pore postero-dorsal; bladder a slender, median tube extending anteriorly to level of ovary where right and left primary collecting ducts enter.

Mean measurements in millimeters (with minima and maxima within parentheses) of five adult worms mounted in toto: Body, length 1.766 (1.475–1.995), width at intestinal bifurcation 0.288 (0.248–0.330), at anterior testis 0.738 (0.540–0.915); oral sucker, length 0.258 (0.195–0.300), width 0.228 (0.195–0.255); acetabulum, 0.188 (0.155–0.218) \times 0.194 (0.180–0.225); esophagus, length 0.173 (0.160–0.180); left vitellarium, 0.144 (0.136–0.152) \times 0.081 (0.056–0.099); right vitellarium, 0.130 (0.101–0.149) \times 0.083 (0.067–0.136); ovary, 0.146 (0.117–0.173) \times 0.139 (0.110–0.165); anterior testis, 0.148 (0.120–0.220) \times 0.122 (0.101–0.147); posterior testis, 0.178 (0.140–0.225) \times 0.129 (0.106–0.165).

Hosts and localities: *Etheostoma blennioides blennioides* Rafinesque from Huron and Saline Rivers; *Hadropterus maculatus* (Girard) and *Percina caprodes semifasciata* (De Kay) from Honey Creek; all in Washtenaw County, Michigan.

Habitat: Urinary bladder.

Type: U. S. Nat. Mus. Helm. Coll. No. 36820 (from *Etheostoma b. blennioides*, Saline River); paratypes in author's collection.

Of eight *P. etheostomae* examined, five had the ovary on the left and three on the right indicating that amphitropy occurs frequently in this species.

In possessing an oral sucker larger than its acetabulum *etheostomae* differs from all known species of *Phyllodistomum* with the exceptions of *brevicicum* Steen (1938), *caudatum* Steelman (1938), *mogurndae* Yamaguti (1934), *pearsei* Holl (1929) and *unicum* Odhner (1902). This species differs from *brevicicum* in possessing two pairs of marginal folds instead of a single pair, and from the other four which have no marginal folds. Of the six species compared, *etheostomae* and *mogurndae* have the posterior end of the body notched; *etheostomae* lacks the caudal projection characterizing *caudatum*. The present species is distinguished also in having its intestinal crura end at the posterior fourth of the body instead of at the posterior testis as in *brevicicum*, or near the posterior end of the body as in the other four species. It differs further from *caudatum* and *unicum* in having its uterus extensively developed extracurally as well as intracurally. It is distinguished further from *pearsei* in having sucker ratios of 1:0.85 \times 1:0.74 instead of 1:0.59, and its testes are approximately twice as long as in *pearsei*, although their widths are similar. In having its body and eggs significantly smaller *etheostomae* is differentiated further from *mogurndae* and *unicum*.

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THE LIFE CYCLE OF FOUR INTESTINAL COCCIDIA OF THE DOMESTIC RABBIT

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Although infections with coccidia have long been recognized among domestic and game animals, considerable confusion still exists concerning classification of various species of coccidia, their life cycles and methods of control. Coccidia are of great economic importance to animal industry. Zoologists, pathologists and investigators in related fields have contributed information toward the control of these parasitic protozoa and one of the factors which aids in an intelligent interpretation of this problem is derived from a study of life cycles. While the control of coccidiosis is not entirely dependent upon a definite knowledge of the life cycles, such information does add appreciably, and in turn aids in instituting prophylactic measures.

In the domestic rabbit, *Oryctolagus* (Hobbs, 1931), five species of *Eimeria* occur. Four of these multiply in the epithelial cells of the small intestine and one in the epithelium of the bile passages. Early workers in this field failed to differentiate all four of these species because they often dealt with mixed infections. Kessel and Jankiewicz (1931) clarified the situation by differentiating all four on the basis of the morphological structure of their oöcysts. Accepting this differentiation it has been possible to segregate the four species, and establish pure infections of each in coccidia-free rabbits, thus enabling one to determine their endogenous life cycles. The species which is parasitic in the bile ducts was first noticed by Leeuwenhoek in 1674. He described bodies in the bile of rabbits which Dobell (1922) believes were the oöcysts of *Eimeria stiedae*. Hake (1839), according to Wenyon, first described the pathology of coccidiosis in the liver and duodenum of the rabbits, although he believed the oöcysts to be "pus globules" associated with carcinoma of the liver. No attempt was made, however, by the first investigator to differentiate between the species inhabiting the liver and intestine.

It was not until 1879 that Leuckart proposed the name *Coccidium perforans* for the coccidia he found to inhabit the intestine, and it is assumed that he described, for the most part, the species now known as *Eimeria perforans*.

Remak (1845) first noted oöcysts in the intestinal mucosa and Kauffmann (1847), according to Wenyon, observed that the oöcysts segmented into four separate sporocysts if kept in water. Stieda (1865) corroborated this observation and noticed in addition that within each sporocyst two elongated structures developed. These are now known as sporozoites. Balbiani (1884) proved that the sporocysts produce two sporozoites and a small residual body. It was Lindemann (1865), however, who recognized the parasitic nature of these cysts and called them *Mono-cystis stiedae*. In 1875 Schneider established the genus *Eimeria* for the coccidium of the mouse, and Leuckart's genus, *Coccidium*, is now considered a synonym for *Eimeria*.

L. Pfeiffer (1890a, 1891) and R. Pfeiffer (1892), according to Wenyon, were the first to indicate that an endogenous multiplication occurred in the rabbit's in-

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testine from which oöcysts developed. Simond (1897) fed oöcysts to young rabbits, reared from birth on sterile milk, and demonstrated the intermediate stages of schizogony, as well as newly formed oöcysts like the original ones fed.

Waworuntu (1924) described certain developmental stages of *E. perforans* and also a few for *E. stiedae* while Perard (1924a) also gives a few stages of development of *E. perforans* and *E. stiedae*. In 1924a, Perard reported a new intestinal species which in 1925b he named *Eimeria magna*. Prior to his description, other workers had considered this to be a larger form of *E. perforans* or had confused it with *E. stiedae*.

Waworuntu (1924) failed to differentiate between these three species and produced intestinal coccidiosis with what he thought to be *E. stiedae*. In reality he was working with *E. magna* as is shown in Plate II, Fig. 9 of his publication. Wenyon (1926) and Krijgsman (1926) also confused *E. magna* and *E. stiedae*.

Waworuntu (1924) reported a 3rd type of oöcyst, intermediate in size between *E. stiedae* and *E. magna*, which is really smaller than *E. magna*. This new type also possessed morphological differences and was named *E. media* by Kessel and Jankiewicz (1931). These workers named an additional species found in the intestine, *E. irresidua*, the oöcyst of which is easily confused with *E. stiedae*.

It is the purpose of the work here reported to determine in detail the endogenous phases of the life cycle of these four species and to correlate them with the exogenous phases previously described.

MATERIALS AND METHODS

The oöcysts of each of these four species of coccidia are easily differentiated by their characteristic appearance. Since as many as three species have been found to occur at one time in a naturally infected rabbit, the first problem was to isolate pure lines. This was accomplished in a variety of ways after the length of the life cycle for each species had been determined.

E. magna was the first one to be obtained free from the other species. This was accomplished by feeding to young coccidia-free rabbits, sporulated oöcysts of *E. magna* with which were found a few *E. perforans*. Pellets containing oöcysts were collected from these animals, after seven days, and allowed to sporulate for a period of from 4 to 5 days in shallow dishes to which was added sufficient 2% potassium dichromate solution to keep the feces covered. This procedure was repeated three times, and on each occasion the newly collected oöcysts were used to establish the new infection. After the 3rd successive feeding of the sporulated material, a pure strain of *E. magna* was obtained.

From a rabbit which was infected with both *E. magna* and *E. irresidua*, in which *E. irresidua* predominated, sections of the intestine were taken at intervals of three inches and examined for oöcysts by microscopic examination of the mucosal scrapings. Oöcysts of *E. irresidua* only were found to occur in the first six inches of the intestine, whereas in the lower regions they were found to occur together. These oöcysts of *E. irresidua* were allowed to sporulate and upon continued infection gave a pure line.

E. magna and *E. media* often occur together in infected rabbits. The former has a developmental cycle of seven days, while *E. media* requires but six days. By feeding a mixture of these species and by collecting pellets on the 6th day of infection, one is able to obtain oöcysts of *E. media* only. By removing all food on the 4th day

after administering the infective dose and then returning it between the 5th and 6th days, showers of oöcysts of *E. media* will be discharged.

E. perforans completes its life cycle in five days. By following the procedure as outlined above for *E. media*, one is able to obtain a pure strain of this small species.

After the desired species were obtained in pure form, they were routinely passed through two successive rabbits as a check against contamination. These oöcysts from pure species were collected either from cecal contents or the pellets, sporulated and fed to the coccidia-free animals used for the life cycle studies.

Before feeding sporulated oöcysts, contained in fecal material saturated with potassium dichromate, one must remove this chemical. This is easily accomplished by filling centrifuge tubes one-fourth to one-third full with feces, adding water, stirring, and centrifuging. All oöcysts and debris collect in the bottom and the supernatant fluid can be poured off. By adding a small amount of water to the sediment, the debris is easily removed from the centrifuge tube.

It was found that the slow passing of air or oxygen through the dichromate mixture of the feces insured the sporulation of a maximum number of oöcysts in the shortest possible time.

All rabbits used in these experiments were raised in separate isolation rooms. Pregnant does were separated from one another by placing them in sterilized wire cages which for added precautionary measures were changed every 48 hours. Food containers were also sterilized and changed with each exchange of cages.

After the young were a week old their nesting boxes were also changed every two days. The young were taken from their mothers, when 21 days old, and placed in small, sterilized, isolated cages in a separate room. These animals were checked every day for a week before infection and every day thereafter until the animals were sacrificed.

Infection was effected, in most cases, by injecting oöcysts directly into the stomach by means of a Luer syringe to which was attached a piece of small rubber tubing. Animals which were to be sacrificed during intervals of the first three days, were given a larger number of oöcysts than those infected for longer periods. This insured the presence of many more stages in the tissues for study.

An animal from each feeding experiment was sacrificed each 12 hours for the first three days and thereafter every 24 hours up to the 12th day. Tissues for histological studies were taken from 4 to 6 different regions of the intestine.

All tissues were fixed in Bouin's fluid, dehydrated, embedded in paraffin, and sectioned. After several stains were tried, it was decided that iron haematoxylin was superior to others. This was also employed for smears which were made from many regions of the intestine at the same time the tissues were taken.

All drawings were made in ink with the aid of a camera lucida, from stages found either in the tissues or on smears. The drawings were made to an original magnification of $\times 1700$ except Figs. 31, 68, 91 and 117, which were $\times 2700$.

EXPERIMENTAL RESULTS

Eimeria irresidua

The development of the intestinal parasite, *Eimeria irresidua*, Kessel and Jankiewicz (1931), occurs in the epithelium of the villi, from the duodenum to the lower ileum. The heaviest infection found was located in the first 18 inches of the gut

before the liberation of the 1st generation of merozoites. After these are freed, 2nd generation schizonts may develop at any place within the entire length of the gut.

Since this study deals with the endogenous cycle of this parasite, a suitable point at which to begin the description is immediately after excystment of the sporozoites in the intestine.

Rabbits killed after an infection of six hours show sporozoites (Figs. 1-3) free in the lumen and young schizonts developing in the epithelium. Sporozoites appear to remain in an infective condition in the intestine for at least three days, since tissues taken at this time may show sporozoites that have recently entered the host cells.

Sporozoites of this species are small compared with similar stages found in other *Eimeria*. They attain a small, spherical structure six hours after being freed from the oöcyst and measure 2.8μ in diameter. They possess a large granule, the 'hyaline globule' of some authors, and a smaller one, which is perhaps the karyosome of an indistinct nucleus. Both of these stain black with iron haematoxylin.

Sporozoites (Figs. 3 & 4) that have just entered the epithelium, elongate slightly, causing the two granules to move further apart. All of these forms are now 2.5 to 3.5μ in length and occur mostly in the distal or lumen end of the host cell, although some have migrated past the nucleus toward the cell base. Twelve hours later many more of the developing schizonts are located in the proximal or basal region of the cell (Fig. 4).

These stages (Figs. 4 & 5), have now become 3.0 to 5.7μ in diameter, with many showing an increase in their number of nuclei. The nuclear membranes are indistinct, comparing favorably with the observations of Tyzzer (1929) in his work on the coccidiosis of chickens.

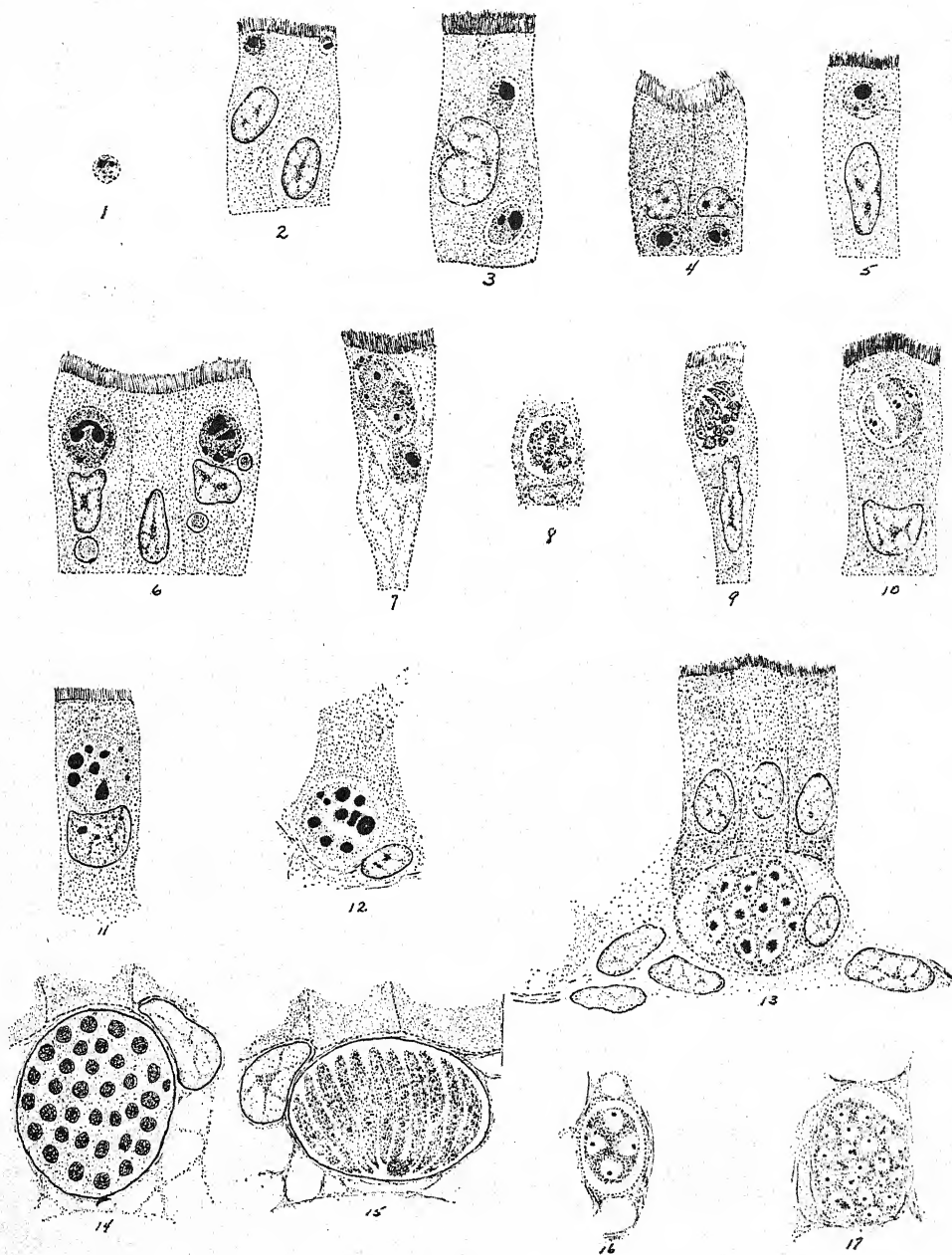
The stages which remain on the distal side of the nucleus, develop into a generation of small merozoites.* These pockets are small and contain few individuals. The ones at the base of the cells are larger and many more merozoites are contained in each pocket. Both of these schizonts mature at the same time. To avoid confusion, the smaller ones which are also fewer in each cell are designated as type A

TABLE 1.—Summary of relationships of the merozoites in four species of intestinal coccidia of the domestic rabbit

	Species											
	<i>E. iryesidua</i> (9-10)*			<i>E. magna</i> (7)*			<i>E. media</i> (6)*			<i>E. perforans</i> (5)*		
	Size (μ)	Days dev.	No. in 1 schizont	Size (μ)	Days dev.	No. in 1 schizont	Size (μ)	Days dev.	No. in 1 schizont	Size (μ)	Days dev.	No. in 1 schizont
1st generation type A	1.7 x 7.0	6	2-8	2.8 x 8.5	4	2-10	1.5 x 6.7	4	2-10	1.5 x 5.7	4	2-8
2nd generation type A	2.0 x 7.2	9	8-10	3.0 x 10.0	6	4-16	2.0 x 8.0	6	2-10	1.4 x 5.7	5	2-8
1st generation type B	1.8 x 11.5	6	36-48	2.0 x 8.5	4	40-60	1.4 x 4.6	4	12-36	1.0 x 4.3	4	8-32
2nd generation type B	1.3 x 7.2	9	48-76	1.5 x 7.2	6	48-78	1.2 x 4.3	6	24-36	1.2 x 4.3	5	16-30

* Indicates the days required for completion of the life cycles.

* Table 1 gives in tabular form a summary of the relationship of all the species discussed in this paper.

*Eimeria irresidua*

- FIG. 1. A sporozoite drawn from a smear six hours after infection, 2.8 μ .
 FIG. 2. Sporozoites after entering a host cell, six hours.
 FIG. 3. Young schizonts in the proximal and distal portions of a cell, 3-4.3 μ .
 FIG. 4. Young schizonts in the proximal region of a cell, 3 μ , 12 hours.
 FIG. 5. Young schizont showing nuclear structure, 5 μ , 24 to 36 hours.
 FIG. 6. Young schizont showing nuclear structure and food vacuoles, 7 μ , 80 hours.
 FIG. 7. Young schizont, 10 μ , four days.
 FIG. 8. Developing schizont of 1st generation, type A, 7.2 \times 8.5 μ , six days.
 FIG. 9. Immature merozoites or type A, pocket 5 \times 10 μ , six days.

and the larger ones as type *B*. As schizonts designated as type *B* grow, many push past the basement membrane with their host cells bulging into the subepithelial layers.

Both types of schizonts, *A* and *B*, continue to grow rather slowly when compared with species in other animals. After 36 hours one finds them 4.5 to 7.2 μ in diameter (Fig. 5), each containing from 2 to 6 nuclei, karyosomes, and the hyaline globule.

During the 4th day one observes that the two types of schizonts exhibit signs of differentiation (Figs. 6, 7, 8, 11, 12 & 13), the ones in the distal end of the cells, the type *A*, being smaller and containing fewer nuclei than the type *B*.

After the 6th day mature merozoites (Figs. 9, 10, 14 & 15) are seen in both locations and are interpreted as representing the 1st generation, since they mature approximately at the same time. Mature pockets in the epithelium on the distal end (*A*) develop into schizonts containing from 2 to 10 banana-shaped individuals, pointed at both ends, with four as the average number present.

These merozoites are 1.5 to 1.8 μ in width by 6.5 to 7.2 μ in length. The nucleoplasm is clear except for two dark granules, one being slightly larger than the other. The nucleolus is bounded by a distinct membrane.

Between the 4th and 6th days *B* schizonts increase in size and show a multiplication of their nuclear elements. When mature (Figs. 14 & 15) these pockets are 13 by 17 μ in size and contain from 36 to 48 merozoites. These curved merozoites average 1.8 by 11.5 μ in their measurements. In the pointed extremity of most of these one finds small, darkly staining granules, while the blunt end is devoid of any such structure. The nucleus is somewhat excentrically situated toward the pointed extremity and has a prominent karyosome and a definite nuclear membrane.

The liberation of these merozoites is made possible by a gradual necrosis of the tip of the villi. These individuals are then free to produce their 2nd generation schizonts.

The developing 2nd generation merozoites derived from the 1st generation schizont *B* are located in regions similar to those for the early macrogametocytes.

The other generation of merozoites, developing at the same time as those above, are much more common and persist to the 10th day of infection. Their earlier stages (Figs. 16 & 17) present pockets showing 4 to 30 nuclei, each containing a delicate karyosome surrounded by a clear area and an indistinct limiting membrane. Later stages (Fig. 18) indicate that merozoites develop from a central mass of cytoplasm. The mature pockets contain from 48 to 75 merozoites (Figs. 19 & 20) measuring 1.3 by 7.2 μ .

Early stages (Fig. 23), showing 2 to 4 karyosomes, are found on the 6th day, and by the 7th to 8th day, one finds pockets showing 8 to 12 nuclear divisions (Fig. 24). The dense nuclear or karyosome material in the more developed schizonts

FIG. 10. Mature merozoite of 1st generation, type *A*, 1.5–1.8 \times 6.5–7.2 μ , six days.

FIG. 11. Young schizont showing nuclear differentiation, 7.5 μ , 80 hours.

FIG. 12. Young schizont showing differentiation, 8.5 μ , four days.

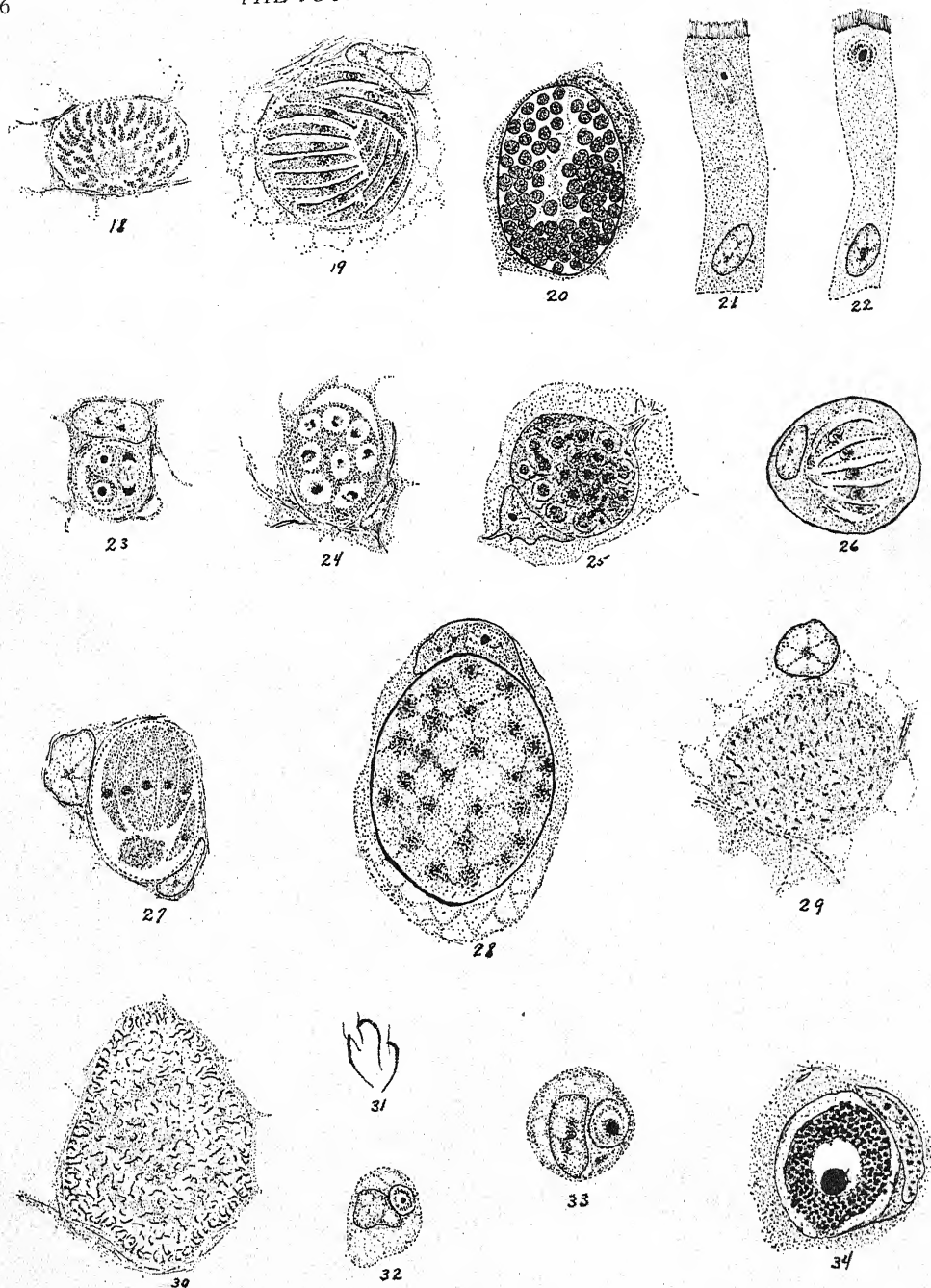
FIG. 13. Greatly enlarged schizont of 1st generation, type *B*, located in subepithelial area, six days.

FIG. 14. Cross section of mature merozoites of the 1st generation, type *B*, pocket 14 μ , approximate number 36, six days.

FIG. 15. Mature merozoites of 1st generation, type *B*, a small residual mass is present, 1.8 \times 11.5 μ , six days.

FIG. 16. Early 2nd generation schizont, type *B*, 5.7 \times 8.5 μ , eight days.

FIG. 17. Second generation schizont, type *B*, 10 \times 15.7 μ , eight days.

*Eimeria irresidua*

- FIG. 18. Immature 2nd generation schizonts, type B, pocket $10 \times 14 \mu$, nine days.
 FIG. 19. A pocket of mature 2nd generation merozoites, type B, individuals $1.3 \times 7.2 \mu$, 9 to 10 days.
 FIG. 20. Cross section of mature 2nd generation merozoites, type B, pocket $13 \times 18.5 \mu$, number 78, 9 to 10 days.
 FIG. 21. A merozoite of type A, 1st generation, after entrance into a new host cell, six days.
 FIG. 22. Young schizont, 2nd generation, type A.

seem to have a spindle-like arrangement extending from one pole out into the clear area surrounding the centriole. This extension, which gives it a brush-like appearance, is finely granulated in places and corresponds to the description given by Tyzzer (1929) of *E. tenella* for a growing 2nd generation schizont.

The mature merozoites (Figs. 26 & 27) of this generation are only slightly curved, measure 2.0 by 7.2 μ , are pointed at both ends and have a nucleus situated slightly excentrically. These pockets occur less frequently than do those of type A and contain from 8 to 16 merozoites.

As young microgametes undergo division, the entire gametocyte increases in size. By the 8th day microgametocytes (Fig. 28) are easily distinguished from the early developing merozoites. These developmental stages of the nuclei are large, diffuse masses connected by strands and are evenly distributed throughout the gametocyte. By the 9th day metamorphosis of the microgametocytes is nearing completion; pockets containing mature microgametocytes (Figs. 29 & 30) are present, and are quite evenly distributed.

Since early macrogametocytes (Figs. 32 & 33) are found in host cells closely associated with the liberated B merozoites, it appears that oöcysts are derived from type B of the 1st generation. Later young oöcysts occur at the base of the epithelial cells in close association with connective tissue elements as were the 1st generation type B merozoites. After the 8th day the gametes and merozoites do not appear to be limited to any particular region of the mucosa or upper submucosa.

Early macrogametocytes (Fig. 32), 3.5 to 4.5 μ , are spherical and contain a central karyosome and possess an indefinite nuclear border with little cytoplasm. By the beginning of the 8th day (Fig. 34) they are much larger, with the average diameter 14 to 16 μ . Their cytoplasm is granular and contains a nucleus 2.8 to 3.0 μ with a large karyosome, surrounded by a clear area.

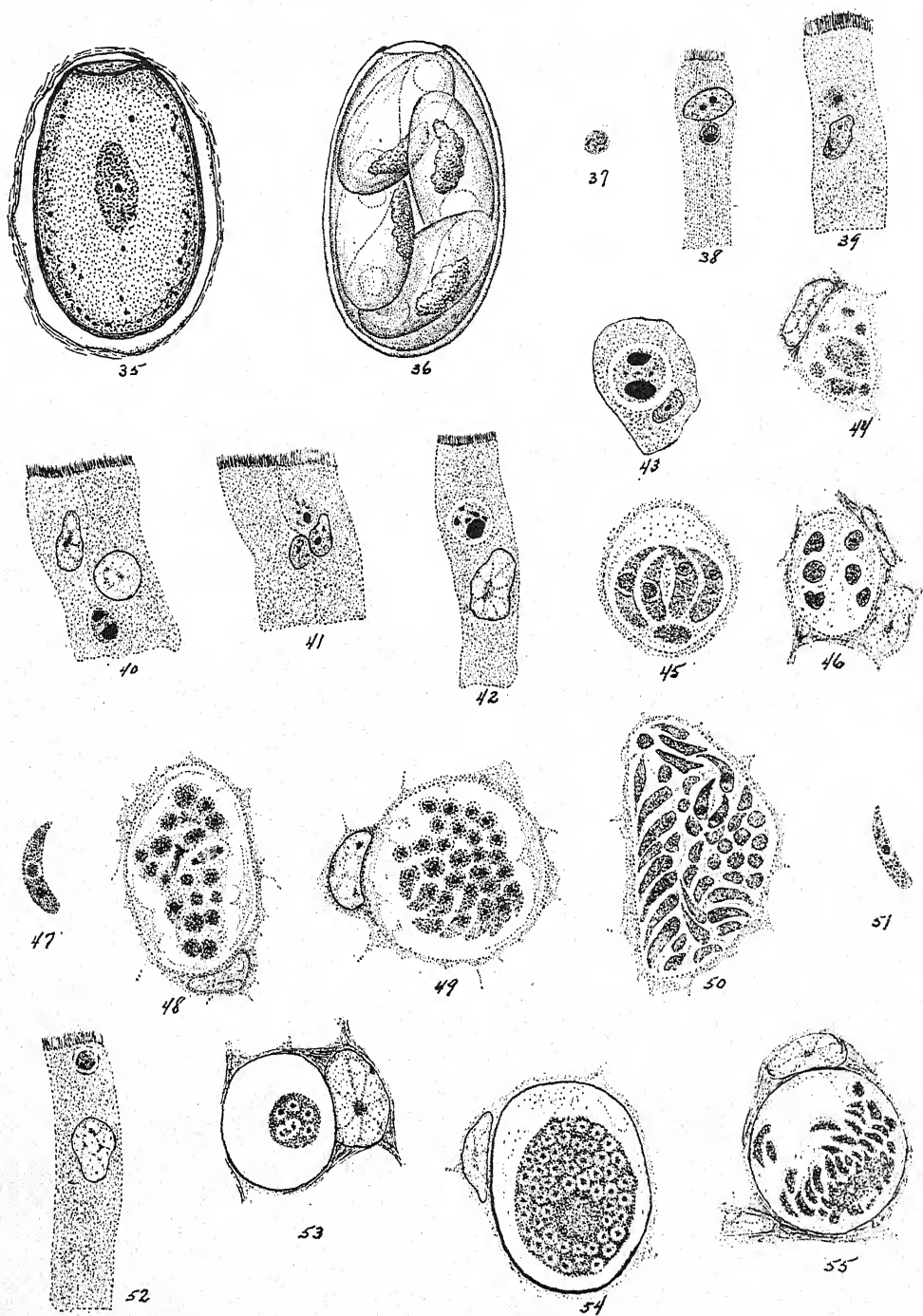
During the 8th and 9th days well developed oöcysts (Fig. 35) are seen. Each has a centrally located nucleus and surrounding cytoplasm which fills the entire cell. At the periphery are globules of material which aid in forming the cyst wall. A section through the micropyle shows the typical character of these oöcysts of *E. irresidua* (Figs. 35 & 36).

Eimeria magna

Eimeria magna, Perard (1925a), is the only one of the four species which has not been found to develop in the upper small intestine. It favors the middle jejunum and all of the ileum, requiring seven days to pass through its endogenous period.

Twelve hours after an infective feeding of oöcysts sporozoites (Figs. 37 & 38)

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- FIG. 23. Early 2nd generation schizont, type A, showing division stages, six days.
FIG. 24. Second generation schizont, type A, pocket 8.5 \times 11.5 μ , eight days.
FIG. 25. Second generation schizont, type A, division stage present, pocket 11.5 μ , eight days.
FIG. 26. Nearly mature 2nd generation, type A.
FIG. 27. Mature 2nd generation, type A, merozoites, individuals 2 \times 7.2 μ , nine days.
FIG. 28. Young microgametocyte showing diffuse masses still in the process of division, pocket 18 \times 28 μ , eight days.
FIG. 29. Microgametocyte showing young microgametes, pocket 15.7 \times 18.5 μ , nine days.
FIG. 30. Microgametocyte showing mature microgametes, pocket 20 \times 28.5 μ , nine days.
FIG. 31. Microgametes \times 2700.
FIG. 32. Young macrogametocyte, 4.3 μ , six days.
FIG. 33. Young macrogametocyte, 4.3 \times 5.7 μ , six days.
FIG. 34. Growing macrogametocyte, 14 \times 16 μ , eight days.

*Eimeria irresidua*

- FIG. 35. Macrogametocyte ready to be discharged from host cell, $24 \times 36 \mu$, nine days.
 FIG. 36. Fully sporulated oöcyst.

Eimeria magna

- FIG. 37. Sporozoite from an intestinal smear 12 hours after an infective feeding.
 FIG. 38. Young schizont, 2.0μ , 12 hours.

are found to have just entered their host cells. Young schizonts (Figs. 39, 40, & 41) are found by the 36th hour of infection. These larger schizonts show 1 to 2 nuclei and average $3.5\ \mu$ in diameter. During the next 24 hours the schizonts double in size as the nuclei increase in number (Fig. 42) and by the 3rd and 4th days still larger schizonts are observed showing continued nuclear divisions (Figs. 43 & 44). On the 4th day young and adult merozoites will be seen which belong to two distinct types previously mentioned as occurring in *E. irresidua*. Both of these types should be considered 1st generation merozoites. Schizonts of the type *A* (Figs. 45 & 46) merozoites measure 10 by $14\ \mu$, and contain from 2 to 10 individuals which are 2.8 by $8.5\ \mu$ when mature. They are curved, pointed at one end and blunt at the other, with a nucleus that is usually centrally located. These merozoites immediately enter other host cells to become either 2nd generation schizonts or develop into gametes. The 2nd generation schizonts soon begin nuclear division if they are to undergo schizogony and become 2nd generation merozoites.

The other types of merozoites which are here designated as *B*, 1st generation (Figs. 48–51), are smaller, containing from 40 to 60 in a pocket; they are slightly curved, pointed at one end and rounded at the other, and measure 2.0 by $8.5\ \mu$. These have a central nucleus with a pronounced clear area about the pole toward the rounded end. They may produce either gametes or another generation of merozoites.

Fig. 52 shows, what appears to be, a rounded merozoite in a host cell which probably develops into the generation of merozoites which is destined as 2nd generation type *B*. On a slide prepared after 5 to 6 days infection one finds schizonts containing 6 to 75 immature merozoites (Figs. 53 & 54). More advanced merozoites are seen in association with the central mass (Fig. 55) as well as mature ones (Fig. 56). Pockets containing these adult individuals with their components measure 19.5 by $21.4\ \mu$.

Figs. 57–62 show phases in the progressive maturation of 2nd generation *A* schizonts containing few, large individuals. These, when mature, average maximum lengths of 3.0 by $10\ \mu$. They are pointed at one end and blunt at the other with a

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- FIG. 39. Young schizont, $2.8\ \mu$, 24 to 36 hours.
 FIG. 40. Young schizont, $3.5\ \mu$, 36 hours.
 FIG. 41. Young schizont, $3.5\ \mu$, 36 hours.
 FIG. 42. Young schizont showing nuclei, $4.3\ \mu$, 60 hours.
 FIG. 43. Young schizont showing nuclei, $7.5\ \mu$, 80 hours.
 FIG. 44. Young schizont, 1st generation, type *A*, $7.2 \times 10\ \mu$, four days.
 FIG. 45. Mature merozoites, 1st generation, type *A*, individuals $2.8 \times 8.5\ \mu$, four days.
 FIG. 46. Cross section of pocket containing six merozoites of 1st generation, type *A*, pocket $10 \times 14.4\ \mu$, in six days.
 FIG. 47. A 1st generation, type *A*, merozoite, $2.8 \times 8.5\ \mu$, four days.
 FIG. 48. Young schizont of 1st generation, type *B*, $13 \times 21.5\ \mu$, 3 to 4 days.
 FIG. 49. Young schizont of 1st generation, type *B*, showing one of the merozoites of elongation, $18\ \mu$, four days.
 FIG. 50. A pocket of nearly mature merozoites, $17 \times 24\ \mu$, four days.
 FIG. 51. Mature 1st generation, type *B*, merozoite $2.0 \times 8.5\ \mu$, four days.
 FIG. 52. A 1st generation, type *B*, merozoite after entering a new host cell, destined to become a 2nd generation, type *B*, schizont, $2.8\ \mu$, four days.
 FIG. 53. Second generation, type *B*, schizont, pocket $8.5 \times 10\ \mu$, five days.
 FIG. 54. Second generation, type *B*, schizont showing immature merozoites coming from a central mass, $13.0 \times 18.5\ \mu$, six days.
 FIG. 55. Young merozoites of 2nd generation, type *B*, some are still clinging to a central mass, pocket $17.0\ \mu$, six days.

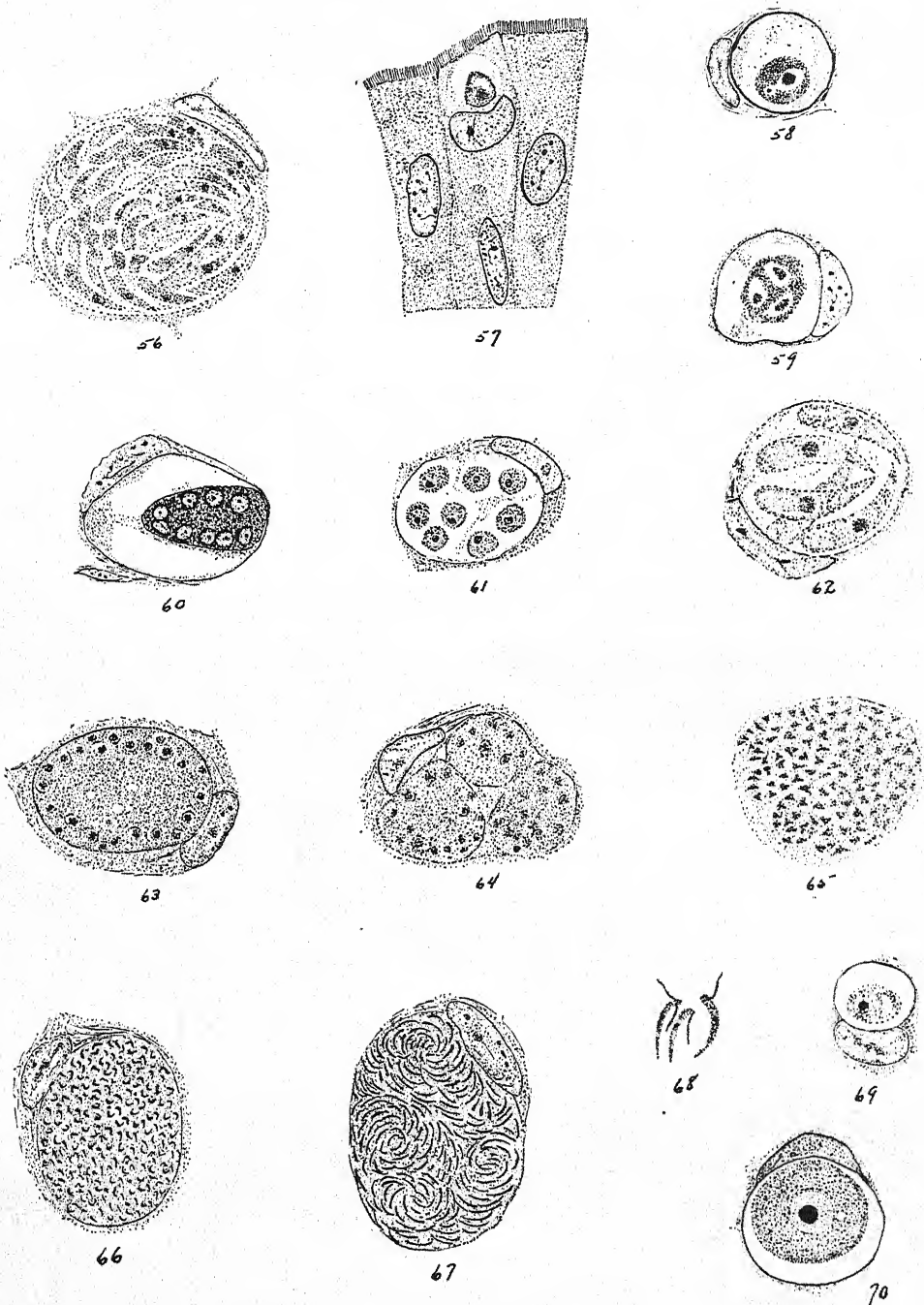
*Eimeria magna*

FIG. 56. Mature pocket of 2nd generation, type B, merozoites, pocket $18.5 \times 21.4 \mu$, individuals, $1.5 \times 7.2 \mu$, six days.

FIG. 57. Young schizont of 2nd generation, type A, six days.

FIG. 58. Young schizont, type A, showing 1st division, six days.

FIG. 59. Young schizont, type A, showing three nuclear structures, $5.7 \times 7.2 \mu$, six days.

FIG. 60. Young schizont, 2nd generation, type A, showing eight nuclear divisions, surrounding a central mass, $7.2 \times 13 \mu$, six days.

centrally located nucleus. There are from 4 to 16 individuals in a pocket. Merozoites of this type are the largest encountered in any of these species.

It is impossible to distinguish the early gametes from one another, but on the 5th day, one observes stages (Fig. 63) which are destined to become microgametocytes. In these pockets the nuclear structures undergo a large number of divisions while the cytoplasm increases in volume. These small nuclear stages (Fig. 64) are but $1\ \mu$ in size and appear more definite than in other species. During their transformation they pass successively from triangular (Fig. 65) to comma-shaped individuals (Figs. 66 & 67), measuring 0.5 by 2.5 to $3.0\ \mu$. Many of these microgametocytes appear lobulated, caused by an infolding of their surfaces (Fig. 64). The fully developed microgametocytes appear in whorls and are quite evenly distributed throughout the gamete although in some pockets they tend to collect toward the periphery. Mature microgametocytes show terminal flagella, and what appears to be a nucleus (Fig. 68) located in the mid-region.

The early, spherical macrogametes (Fig. 69) are definitely recognized from the 5th to 6th day; the older of these (Fig. 70) possesses a nucleus which contains a prominent karyosome which is surrounded by a fine, granular nucleoplasm and a relatively distinct nuclear membrane. Large, spherical forms (Fig. 71), which measure 18 by $20\ \mu$, contain large plastic globules in their cytoplasm. These are the periphery and elongate as their walls are formed and begin to assume the characteristic morphologic appearance of *E. magna* (Fig. 73) with its typical micropyle. These intracellular stages are 15 to 20 by 30 to $35\ \mu$ in diameter.

Eimeria media

The endogenous phases of the intestinal parasite, *Eimeria media* Kessel and Jankiewicz (1931) take place in the epithelium throughout the entire small intestine. Tissues taken after an infection of from 12 to 24 hours show young schizonts (Fig. 74) in the process of development; some are located distally in the cells while others are located in the proximal regions. During the 2nd day after infection the schizonts (Fig. 75) have increased in size, measuring $5\ \mu$ in diameter and showing on an average six nuclei distributed throughout their cytoplasm. This species exhibits, in its earlier stages, the characteristic hyaline globule that is found in other species. During the next 36 hours the schizonts continue to grow and differentiate into type A and B merozoites of the 1st generation. On the 4th day one finds early

FIG. 61. A cross section of a mature pocket of 2nd generation, type A, merozoites, pocket, $10 \times 14.4\ \mu$, six days.

FIG. 62. Mature merozoites of type A, $3.0 \times 10\ \mu$, these sometimes exhibit a filamentous structure on their narrow extremity, $3.0 \times 10\ \mu$, six days.

FIG. 63. Young microgametocyte showing many rounded nuclear stages, $10 \times 16\ \mu$, five days.

FIG. 64. Later stage of microgametocyte showing infolding of the cytoplasm, $15 \times 17\ \mu$, five days.

FIG. 65. Microgametocyte showing triangular stages of the microgametes, six days.

FIG. 66. Microgametocyte showing elongation of the immature microgametes, $14.4 \times 23\ \mu$, six days.

FIG. 67. Mature pocket of microgametes showing whorl arrangement, pocket, $14 \times 24\ \mu$, individuals, $2.5 \times 3.0\ \mu$, seven days.

FIG. 68. Several mature microgametes showing what appear to be nuclei in their mid regions, $\times 2700$.

FIG. 69. Young macrogametocyte, $3.0 \times 4.3\ \mu$, five and six days.

FIG. 70. Older macrogametocyte, $10\ \mu$, six days.

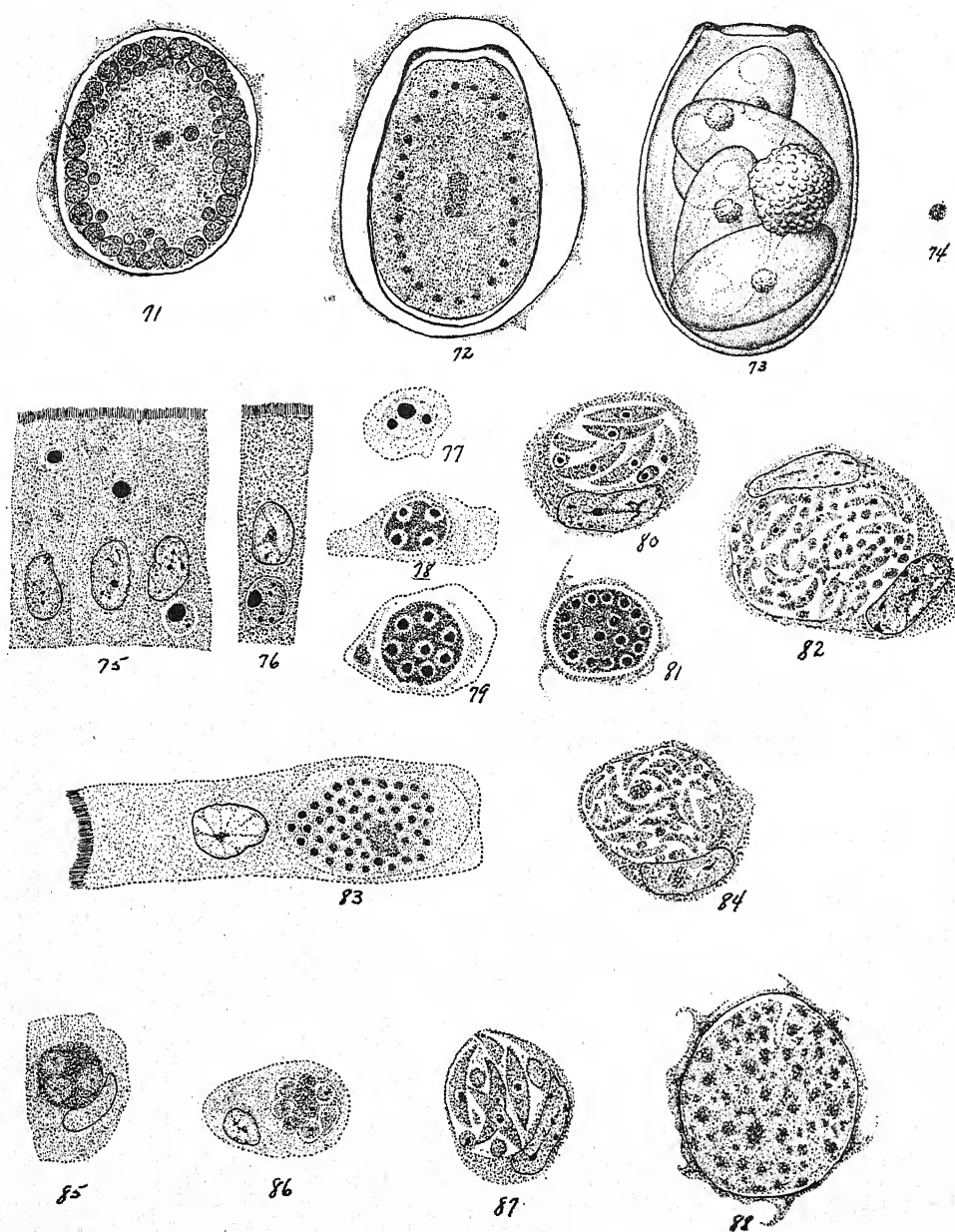
*Eimeria magna*

FIG. 71. Large, spherical macrogametocyte showing "refractile globules" at periphery of cytoplasm, $18 \times 20 \mu$, six days.

FIG. 72. Macrogametocyte showing characteristic micropyle, $15 \times 30 \mu$, seven days.

FIG. 73. Fully sporulated oocyst.

Eimeria media

FIG. 74. Sporozoite from an intestinal smear, $2.0-2.3 \mu$, 12 hours.

FIG. 75. Young schizonts, $2.0-3.5 \mu$, 24 hours.

FIG. 76. Older schizont showing several nuclei, 5μ , two days.

FIG. 77. First generation schizont showing larger nuclei, 6μ , two days.

FIG. 78. Young 1st generation, type A, schizont showing four nuclear structures, $5.0 \times 6.4 \mu$, 3 to 4 days.

stages which represent immature stages of the type destined to become the type *A* merozoites (Figs. 77 & 78). Also at this same time young schizonts (Fig. 79), showing as many as eight nuclear divisions, are present; these nuclear masses stain intensely with iron haematoxylin and are surrounded by clear areas. These structures seem to develop from a common cytoplasmic mass.

Mature merozoites (Fig. 80) derived from these schizonts are present in the same tissue with the previous forms. These merozoites (Fig. 80) derived from these schizonts are present in the same tissue with the previous forms. These merozoites are, for the most part, slightly curved, pointed at both ends and contain a centrally located nucleus which is surrounded by a clear area. They measure 1.5 by $6.0\ \mu$ and are found in pockets containing from 2 to 10 individuals.

Earlier schizonts (Fig. 81) which are the forerunners of type *B* of the 1st generation, are seen during the 4th day. They measure 7.2 by $8.4\ \mu$ and contain many nuclear divisions all embedded in a dark, cytoplasmic material. At this time are found small, mature merozoites (Fig. 82) which are slightly curved, pointed at both extremities and contain a centrally located nucleus. These measure 1.4 by $4.6\ \mu$ and number from 12 to 36 individuals in each pocket.

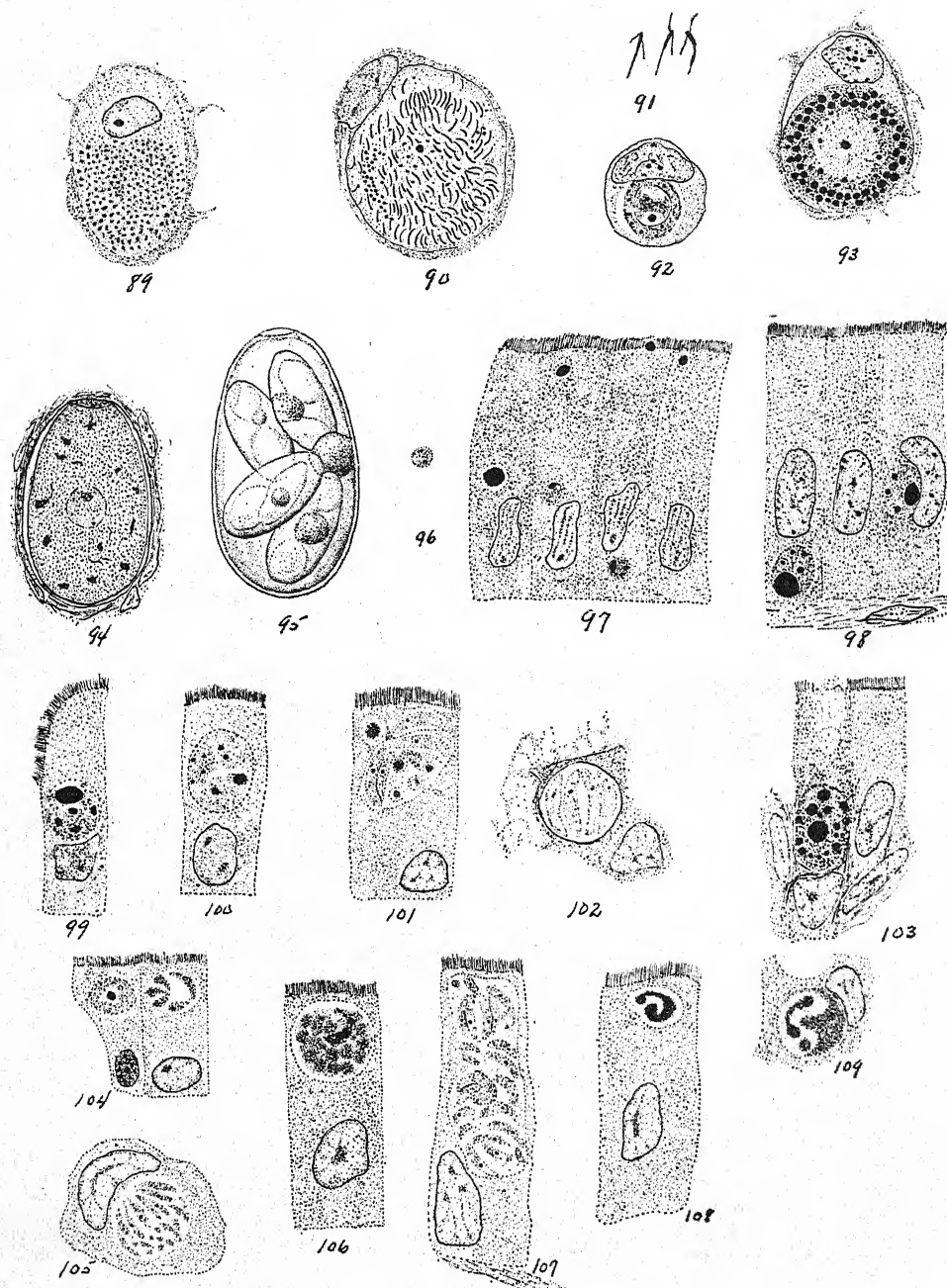
The early 2nd generation type *B* merozoites (Fig. 83), show many nuclear divisions: they are contained in pockets which measure 10 by $17\ \mu$. The adults (Fig. 84), of this type, are 1.2 by $4.3\ \mu$ and are contained in pockets measuring 11.5 by $13\ \mu$. This type of merozoites does not cause as much hypertrophy of its host cells as do those of *E. irresidua* and *E. magna*; they closely resemble *E. perforans* in this respect.

The 2nd generation type *A* merozoites appear on the 6th day and are represented by small schizonts (Fig. 85) containing extremely small nuclei. Later stages (Fig. 86) also appear at this time, showing five or six nuclear divisions, contained in pockets measuring 5.7 by $7.2\ \mu$. Mature merozoites (Fig. 87) measure 2.0 by $8.0\ \mu$, from 2 to 8 individuals occurring in each pocket. They are pointed at both extremities and exhibit a centrally located nucleus.

The metamorphosis of microgametes seems to be similar to that encountered in other species. On the 5th and 6th days one observes microgametocytes (Fig. 88) containing the typical, diffuse nuclear material as well as other stages in which it is condensed (Fig. 89). Pockets containing mature microgametes (Fig. 90) measure approximately $17\ \mu$ in diameter with individuals measuring about $2\ \mu$ in length. The mature microgametes (Fig. 91) have only an indication of a nucleus but do possess flagella.

Macrogametocytes (Fig. 92) also appear on the 5th and 6th days. These are

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- FIG. 79. First generation schizont, type *A*, showing eight nuclear divisions, $7.5\ \mu$, four days.
FIG. 80. Mature 1st generation, type *A*, merozoites, $1.5 \times 6-7.0\ \mu$, four days.
FIG. 81. Early 1st generation, type *B*, schizont, $7.2 \times 8.5\ \mu$, four days.
FIG. 82. Mature 1st generation, type *B*, merozoites, pocket, $18 \times 20\ \mu$, individuals, $1.4 \times 4.6\ \mu$, four days.
FIG. 83. Young 2nd generation, type *B*, schizont, $10 \times 17\ \mu$, six days.
FIG. 84. Pocket of 2nd generation, type *B*, merozoites, pocket, $70 \times 21\ \mu$, individuals, $1.2 \times 4.3\ \mu$, six days.
FIG. 85. Young 2nd generation, type *A*, schizont, $5.7\ \mu$, 5 to 6 days.
FIG. 86. Older stage of developing 2nd generation, type *A*, merozoites, $5.7 \times 7.2\ \mu$, six days.
FIG. 87. Pocket of mature 2nd generation, type *A*, merozoites, $2.0 \times 8.0\ \mu$, six days.
FIG. 88. Early microgametocyte, $21\ \mu$, six days.

*Eimeria media*

- FIG. 89. Later stage of a microgametocyte, six days.
 FIG. 90. Mature microgametocyte, $17.0\ \mu$, individual microgametes, $2.0\ \mu$, six days.
 FIG. 91. Mature microgamete, $\times 2700$.
 FIG. 92. Young macrogametocyte, $5.7\ \mu$, six days.
 FIG. 93. Older macrogametocyte, $14.4\ \mu$, six days.
 FIG. 94. Macrogametocyte ready to be liberated, $14.4 \times 25.7\ \mu$, six days.
 FIG. 95. Fully sporulated oocyst.

small, spherical structures exhibiting the typical dark karyosome. Later stages (Fig. 93) present the usual refractile globules which are lost as the oöcyst wall develops. Oöcysts, ready to be discharged from the host cell, show a zygote surrounded by an evenly staining cytoplasm which fills the entire cyst. This intracellular stage measures 14 by 25 μ . Sporulated oöcysts (Fig. 95) of this species are more variable in their length-breadth ratio than any of the others.

Eimeria perforans

Eimeria perforans Leuckart (1879), is the smallest known parasite of the intestinal group, and has a developmental cycle ranging from five to five and one-half days. It is distributed from the duodenum to the lower ileum.

Sporozoites (Fig. 96) are found free in the lumen a few hours after an infective feeding. They are small, darkly staining, spherical structures 2 μ in diameter. Tissues taken after an infective period of 12 hours, show sporozoites (Fig. 97) that have just entered the epithelium as well as young schizonts, some distal to the nucleus. During the next 12 hours the schizonts (Figs. 98 & 99) double in size while their nuclei increase to three or four. On the 2nd day still larger schizonts are observed showing divisions in their cytoplasm and the presence of six or eight nuclei (Fig. 100). During the later 3rd and early 4th days first generation merozoites are found, which are considered type *A*. The pockets (Figs. 101 & 102) containing these measure 5.7 to 10 μ and contain 4 to 8 individuals which measure 1.5 by 7.2 μ . These are blunt at both ends with a delicate nucleus surrounded by a clear area all located more toward one end than the other.

On the 3rd day of infection, type *B* forms are first distinguished. These stages (Fig. 103) show, on an average, 15 nuclear divisions. During the 4th day these mature and the small pockets show from 8 to 24 merozoites. These are the smallest merozoites (Figs. 104 & 105) encountered in this study; the individuals, measuring 1.0 by 4.3 μ , are contained in pockets measuring 4.3 by 5.7 to 7.0 by 8.0 μ . For the most part they are pointed at both extremities with their nuclei located approximately in the central region.

Intermediate stages in type *B* of the 2nd generation (Fig. 106) have been difficult to locate in this study. Mature pockets of these contain, on an average, 24 mero-

Eimeria perforans

FIG. 96. Sporozoite from an intestinal smear, 2 μ , 12 hours.

FIG. 97. Four epithelial cells showing sporozoites after entering the host cells and young schizonts, 12 hours.

FIG. 98. Schizonts showing nuclei, 4 μ , 24 hours.

FIG. 99. Schizont, 4.3 μ , 24 hours.

FIG. 100. Early schizont of the 1st generation, type *A*, showing cytoplasmic division, 5.7 \times 8.5 μ , two days.

FIG. 101. Mature merozoites of the 1st generation, type *A*, individuals, 1.5 \times 5.7 μ , four days.

FIG. 102. Mature merozoites of type *A*, four days.

FIG. 103. Early schizont of 1st generation, type *B*, 4.3 \times 5.7 μ , three days.

FIG. 104. Schizont of 1st generation, type *B*, merozoite, also a young gametocyte is shown, four days.

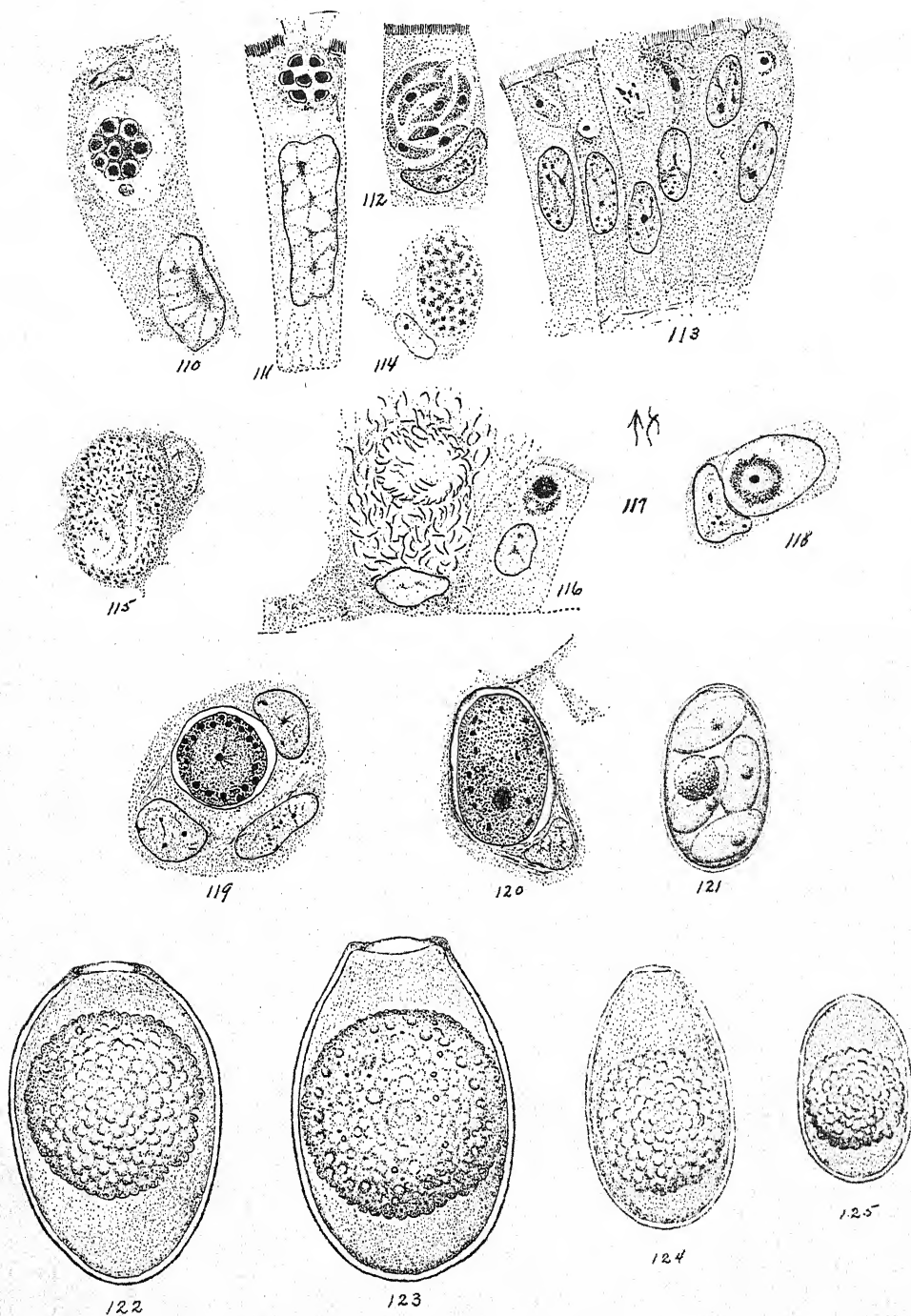
FIG. 105. Pocket of mature 1st generation, type *B*, merozoites, 7.8 μ , individuals, 1.0 \times 4.3 μ , four days.

FIG. 106. Developing schizont, 2nd generation, type *B*, 7.2 μ , four days.

FIG. 107. Mature merozoites of the 2nd generation, type *B*, individuals, 1.2 \times 4.3 μ , five days.

FIG. 108. Early schizont of 2nd generation, type *A*, 4 μ , four days.

FIG. 109. Young schizont of the 2nd generation, type *A*, 5.7 μ , five days.



Eimeria perforans

FIG. 110. Young schizont of type *A* in which the divisions are nearly complete, five days.

FIG. 111. Cross section of a pocket of mature 2nd generation, type *A*, merozoites, 5.7μ , five days.

FIG. 112. Mature 2nd generation, type *A*, merozoites, individuals, $1.4 \times 5.7\mu$, five days.

FIG. 113. Several cells showing stages which result after the entrance of 2nd generation, type *A*, merozoites.

zoites which measure 1.2 by 4.0 μ and have a centrally located nucleus. It is not uncommon, in these mature pockets (Fig. 107) to find groups of merozoites arranged in clusters in one end of the pocket while in the other regions they appear more diffuse in their arrangement. This may result from the development of two schizonts in one host cell.

Type A merozoites, when liberated, enter other epithelial cells and from young schizonts (Figs. 108 & 109) which become 2nd generation merozoites, type A. The pockets of these, which are mature on the 5th day, contain 4 to 8 crescentic individuals (Fig. 110). Their nuclei are large, centrally located and surrounded by a clear area. During development, this generation can be recognized by the density of its chromatin material. These merozoites (Figs. 111 & 112) continue the endogenous cycle as is shown in Fig. 113 which exhibits merozoites penetrating into new host cells to form new schizonts.

Microgametocytes occur during the 4th to 5th day after infection. The earliest stages (Fig. 114) exhibit a diffuse chromatin material. The pockets measure 7.2 by 10 μ . Later stages show small, triangular microgametes (Fig. 115) while the mature microgametes (Fig. 116) are typical, comma-shaped structures which measure approximately 2 μ in length; they are contained in pockets measuring 13 by 17 μ . These microgametes also show a darkly staining protoplasmic mass which suggests a nucleus.

Small macrogametocytes (Fig. 117) are usually observed on the 4th and 5th days. Their development is similar to that described for other species except that they are smaller (Figs. 118 & 119).

E. perforans appears to be the least pathogenic of any of the intestinal species. This is probably due to the production of fewer merozoites.

Frequently the mature oöcyst (Fig. 121) fails to exhibit a micropyle until several days after sporulation is apparently complete.

DISCUSSION

It is now generally recognized that there are five species of *Eimeria* occurring in the rabbit. One of these, *E. stiedae* is found in the liver while the other four are found in the small intestine. Any combination of the four intestinal species may be found in the naturally infected rabbit and can be readily differentiated from each other by a study of the oöcysts. After obtaining the oöcysts of each of the four species in pure form, infecting suitable animals with large doses, sacrificing the animals at appropriate times and preparing histological sections, it is possible to study the endogenous cycles of these *Eimeria*. The author, in this investigation, confirms

FIG. 114. Young microgametocyte, 7.2 \times 10 μ , five days.

FIG. 115. Immature microgametocyte, 10.0 \times 15.7 μ , five days.

FIG. 116. A microgametocyte with mature microgametes; some free. Microgametocyte, 13.0 \times 17.1 μ , microgametes, 2 μ . Neighboring cell shows an aberrant form.

FIG. 117. Mature microgametes, \times 2700.

FIG. 118. Young macrogametocyte, 5.7 μ , 4 to 5 days.

FIG. 119. Older macrogametocyte, 11.5 μ , five days.

FIG. 120. Maturing macrogametocyte, 9.0 \times 17.5 μ , five days.

FIG. 121. Fully sporulated oöcyst.

FIG. 122. Undeveloped oöcyst of *E. irresidua*.

FIG. 123. Undeveloped oöcyst of *E. magna*.

FIG. 124. Undeveloped oöcyst of *E. media*.

FIG. 125. Undeveloped oöcyst of *E. perforans*.

the findings of Kessel and Jankiewicz (1931) by a study of the endogenous phases of these four species. The length of time for completion of these cycles is as follows: *E. irresidua*, 9 to 10 days; *E. magna*, seven days; *E. media*, six days; and *E. perforans*, five days.

Perard (1925a) does not give the length of time required for the endogenous cycle of *E. magna* but states that the schizonts of *E. magna* are oval or spherical measuring 10 to 25 μ with merozoites variable in size measuring 1.0 to 2.5 by 4.0 to 10.0 μ and says that from 2 to 50 merozoites may be present in each schizont. From the above data it appears that he was working with a mixed infection of *E. perforans* and *E. magna*. The larger merozoites reported in his paper (2.5 to 10 μ) correspond favorably with those given here for *E. magna* (3.0 to 10 μ) which occur in the type A 2nd generation. The size of the schizonts and the number of merozoites reported by him from each schizont correspond to those occurring in either of the type B schizonts of the 1st or 2nd generation. The smaller merozoites he reports (1.0 by 4.0 μ) are similar to those found to exist in this work for *E. perforans* (1.0 to 1.2 by 4.3 μ).

In studies on *E. perforans*, Perard reports they have a life cycle requiring four days and develop into a mature spherical schizont measuring 15 μ in diameter and contain "from 30 to 70 long, slender, spindle-shaped, slightly-bowed merozoites, arranged side by side, and each measuring from 10 to 12 μ in length and from 0.5 to 1.75 μ in breadth." Mature schizonts of *E. perforans* in this study measure 4.3 to 10 μ , this size depending upon the type of merozoites contained therein. Merozoites for type A of the 1st generation are 1.5 by 5.7 μ and those of type A 2nd generation measure 1.5 by 6.0 to 7.0 μ , and from 2 to 8 merozoites develop in each cell. The 1st generation type B merozoites measure 1.0 by 4.3 μ and contain from 8 to 32 in each cell, 20 being the average number. The 2nd generation type B measure 1.2 by 4.3 μ , containing 20 to 30 in each cell. Merozoites of the size reported by Perard (0.5 to 0.75 by 10 to 12 μ) have not been found in any of this work, and nothing is mentioned in his work which would indicate the types of merozoites that have been found to occur in this study.

Within a few hours after infecting rabbits with any of these several species, one finds sporozoites free in the lumen and in the intestinal epithelium. These sporozoites seem to remain in an infective condition in the intestine for as long as two days since tissues taken at this time show sporozoites just inside the epithelium. This actually means that younger schizonts are found intermingled with the older stages in the host cells.

The sporozoites encountered on intestinal smears are all small, rounded individuals probably being composed of only nuclear material since no cytoplasm is observed.

Schizonts derived from these develop slowly when one compares them with similar developmental stages in other animals, for example: in *E. irresidua*, which has a cycle of 9 to 10 days, no 1st generation merozoites appear until five or six days after infection while in *E. separata* from the rat, as reported by Roudabush (1937), the 1st generation merozoites are completely formed after 24 hours.

In all species 1st generation schizonts develop in the epithelial cell. When the 1st generation merozoites are liberated they appear to be of two distinct types and are both liberated at approximately the same time. Because this occurs, it is considered

necessary in this work to designate them as type *A* and type *B* of the 1st generation. Type *A* always contain fewer individuals, while type *B* contains many more, except in *E. irresidua* where type *A* merozoites are fewer than type *B*. The size of the merozoites has been shown by other authors but they have failed to designate them as two distinct types.

Merozoites of these types form gametes or 2nd generation merozoites which are designated as *A* and *B* on the basis of size. Second generation merozoites complete their development in from 1 to 3 days depending upon the species.

Gamete formation begins when the 1st generation merozoites enter the host cells. The young gametes are not readily distinguished until the division of their nuclei is discernible. All young gametocytes consist of a central karyosome around which appears a clear area which in turn is surrounded by an area of more dense cytoplasm.

In the development of the microgametocytes, the nucleus undergoes numerous divisions and at the same time the cytoplasm increases in volume. The early chromatin masses are diffuse and connected with one another by fine threads. As development proceeds, these nuclei condense, becoming somewhat pyramidal in shape and as they reach maturity, they become roughly comma-shaped. The fully developed microgametocytes are contained in exceedingly large vesicles often surpassing the macrogametocyte in size but their number is far less.

Young oöcysts are spherical, containing a centrally located karyosome. The cytoplasm of the macrogametocyte contains refractile globules which gradually accumulate at the periphery. These probably serve as reserve food material which aids in the formation of the oöcyst wall. All oöcysts, before their liberation, exhibit a finely granulated cytoplasm and their characteristic micropyle. After the oöcyst is discharged from its exhausted host cell into the lumen, its cytoplasmic mass, which is now the zygote, assumes a spherical shape until discharged with the feces. Outside of the host, under favorable conditions, it will sporulate.

All species, with the exception of *E. magna*, complete their cycles throughout the length of the small intestine. *E. magna* apparently does not inhabit the upper 12 inches of the gut. *E. irresidua* seems to produce the 1st generation of merozoites only in the first 18 to 24 inches of the gut; after liberation of the 1st generation of merozoites, the cycle is completed throughout the entire length of the intestinal tract.

Animals given heavy infective doses of oöcysts show no outward symptoms until approximately 24 hours before the oöcysts are to be discharged, at which time they refuse food and water and appear inactive. These heavy infections give rise to a severe diarrhea which lasts about 12 hours, often resulting in the death of the animal.

E. irresidua and *E. magna* produce the most pronounced pathological changes in the intestinal epithelium. This is probably due to their larger size and greater fecundity as well as their ability to penetrate deeper into the glands while the others remain more in the surface regions and do not seem so prolific. All of these parasites develop within the epithelial cells of the mucosa causing a necrosis of most of the parasitized cells.

The growth of the stages in the cells gradually causes the host cell to enlarge in order to accommodate the parasite. Sometimes the cytoplasm appears only as an outline while in other cases it maintains a considerable volume. The nuclei hyper-

trophy to as much as twice their normal size and appear to be the last cell structure to degenerate.

E. irresidua is the only one of these species which penetrates to the submucosal layers. Epithelium containing stages of parasites are often sloughed into the lumen. This appears to be accomplished by a constriction occurring between the parasitized region and the regenerating epithelium. Denuded areas show various stages of necrosis with the underlying tissues exhibiting active hyperemia and some extravasation of the blood elements.

The majority of *Eimeria* are known only in the oöcyst form. Since many of these resemble one another quite closely it is necessary to rely on their distinguishing characteristics for differentiation. Some of these are measurements, shape, color, cell wall, appearance of micropyle, and the presence or absence of residual bodies.

As the life histories of these different coccidia are discovered, they substantiate the fact that these different oöcysts represent the exogenous stages of specific species.

In the chicken, six species of *Eimeria* are reported, with oöcysts ranging in size from 15.5 by 16.2 to 22.6 by 29.3 μ , while in the rabbit their range is 14.2 by 22.7 to 25.6 by 38.3 μ and in the three species of the rat the range is 16.06 by 13.85 to 24.38 by 22.12 μ . The endogenous cycle of all these *Eimeria* of the chicken and rabbit complete their development in 4 to 7 days, with the exception of *E. irresidua*, which takes 9 to 10 days, and *E. stiedae*, which requires approximately 18 days.

The *Eimeria* of the rat produce 3 to 4 generations of merozoites, while the 1st generation is liberated after an infective period of 24 to 48 hours. A generation is produced every 24 hours thereafter until schizogony is complete. In the chicken the 1st generation of merozoites is liberated between 2½ and 4 days after infection. *E. mitis* of the chicken corresponds more closely to the rabbit coccidia, since its 1st generation merozoites are liberated on the 4th day. In the rabbit the 1st generation merozoites of the intestinal species grow rather slowly and are liberated from 3½ to 6 days after infection, with two distinct types appearing simultaneously; the 2nd generation also produces two distinct types which are liberated at the same time. In *E. perforans* a period of 24 hours occurs between the liberation of the 1st generation merozoites and the production of 2nd generation merozoites. The other intestinal species require 2 to 3 days for the development of similar stages. We thus see that species of coccidia in other animals have generations of merozoites liberated at times intermediate between those required in the rabbit.

The rat has two *Eimeria* parasitizing the small intestine, while a 3rd species requires the cecum and the colon in which to complete its cycle. In the chicken, *E. tenella* has schizonts and oöcysts in the ceca, in *E. necatrix* the schizonts are in the "small intestine," and oöcysts are in the "ceca," with the others favoring the small intestine for their development. Four of the species of the rabbit favor the small intestine, while the 5th lives in the bile ducts.

The above-mentioned species of coccidia from the chicken are the most pathogenic, while in the rabbit *E. magna* and *E. irresidua* appear to cause the most severe infections. All of the *Eimeria* live only in the epithelium of endodermal origin, or in the subepithelial regions or in both locations.

SUMMARY

1. Studies of the endogenous cycles of the four species of intestinal *Eimeria*, found in the domestic rabbit, show that each produces two types of merozoites.

These we may designate as type *A* and *B*, each of which produces a 1st and 2nd generation of merozoites.

2. Merozoites of the 1st and 2nd generations of type *A* of all four species, with the exception of the 1st generation type *A* of *E. irresidua*, are large and contain from 2 to 16 merozoites in each schizont. Those occurring in the 1st and 2nd generation of the type *B* except in the 1st generation of *E. irresidua*, are small and contain from 12 to 78 merozoites in each schizont.

3. It is possible that the 1st generation of type *A* merozoites gives rise to a 2nd generation of type *A* merozoites and microgametocytes, while type *B* merozoites give rise to a 2nd generation of type *B* merozoites and macrogametocytes.

4. Merozoites of the 1st generation of both type *A* and *B* of the four species described require the following lengths of time to mature: *E. irresidua*, 5 to 6 days; *E. magna*, four days; *E. media*, four days; and *E. perforans*, four days.

5. Merozoites of the 2nd generation of both type *A* and *B* of all four species require the following lengths of time to reach maturity: *E. irresidua*, three days; *E. magna*, 2 to 3 days; *E. media*, two days; and *E. perforans*, one day.

6. The four species here reported require the following lengths of time for the completion of their endogenous cycles: *E. irresidua*, 9 to 10 days; *E. magna*, seven days; *E. media*, six days; and *E. perforans*, five days.

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A CONTRIBUTION TO THE BIOLOGY OF *ORNITHODOROS HERMSI* WHEELER, HERMS AND MEYER

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Collections to date indicate that the argasine tick, *Ornithodoros hermsi* Wheeler, Herms and Meyer (Wheeler, Herms, and Meyer, 1935; Wheeler, 1935) is apparently restricted to the mountainous regions of the western United States, specimens having been taken at elevations above 5000 feet from California, Idaho, and Colorado, and at elevations of 3000 and 3600 feet from Oregon.

HABITAT

This species of tick is a parasite of small mammals, particularly rodents, with the most favored host the western chipmunks, *Eutamias* spp. The majority of ticks in California were taken from the nesting material of these animals found in the attics and between the walls of some of the cabins and cottages in several of the mountain resort areas (Herms and Wheeler, 1935). Other specimens have been found in nesting material of chipmunks situated in old hollow logs while several were removed from the sleeping bag of a deer hunter. In Colorado many ticks of this species were collected from chipmunk nesting material in a decaying Douglas fir stump (Davis, 1941). No ticks have been discovered in the nests of the chickaree squirrel, *Sciurus douglasii*, although this species of rodent is another proven reservoir of the spirochetes of relapsing fever. That these squirrels locate their nests high in trees making search extremely difficult in all probability accounts for the fact that no ticks have been taken from the nests of these animals.

METHOD OF REARING

Ticks reared in the laboratory were individually confined in circular pasteboard pill boxes, $1\frac{1}{4}$ by $\frac{3}{4}$ inches. These were arranged in rows on a metal tray constructed from one inch angle-iron to which was soldered a sheet of $\frac{1}{4}$ inch-mesh hardware cloth forming a tray allowing free circulation of air around each of the pill boxes when placed in a temperature and humidity control cabinet (Fig. 1). Warmth was provided by a 60-watt electric lamp, and the temperature was controlled by a mercury column thermostat. Humidity was provided by means of a coarse towel suspended from a wire frame in front of a small electric fan, the free end of the towel being submersed in a shallow pan of water. A hygrothermograph placed on the floor of the chamber gave continuous accurate recordings of both humidity and temperature. After some experimentation it was determined that the ticks developed best at approximately 75° F, and with a humidity of not less than 90 per cent.

FEEDING METHOD

A narrow cylindrical feeding cage fashioned from $\frac{1}{4}$ -inch-mesh hardware cloth was used to confine the mouse while the ticks fed. This cage was made by rolling a piece of the hardware cloth into a cylinder about six inches in length with both ends

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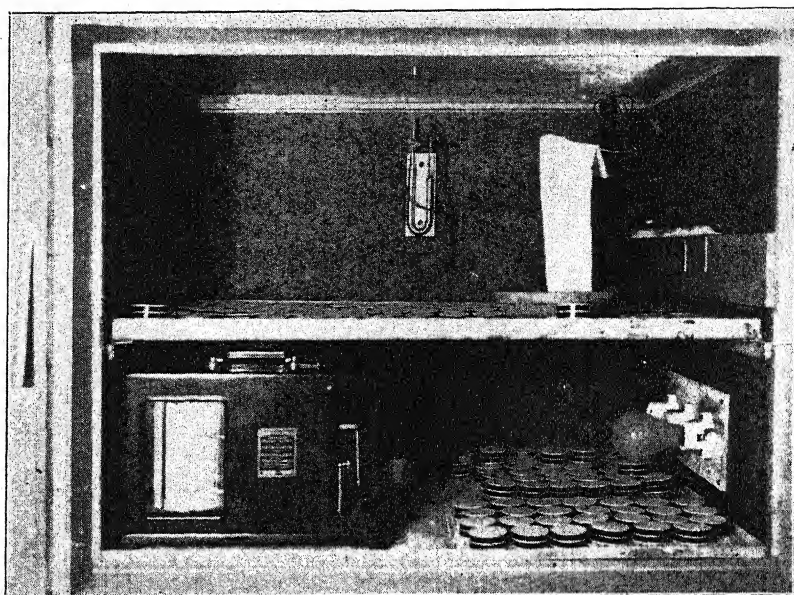


FIG. 1. Constant temperature and humidity control cabinet in which ticks were reared.

open and just large enough to receive a mouse (Fig. 2). An opening one inch long and $\frac{3}{4}$ inch wide was cut in the center of the cylinder to allow for easy access to a portion of the imprisoned animal. The mouse to be used was ushered into the cage head foremost and a plug of cotton forced into the "head end" of the tube as soon as the mouse was in position. A second plug of cotton was then forced into the opposite end of the tube behind the mouse. This cylinder is adjustable to the size of the mouse, and the animal can be easily immobilized by pushing the two plugs of cotton further into the cage. The hair of the mouse was then clipped exposing the skin of the abdomen, and the ticks were applied to the mouse by means of fine pointed forceps. When feeding was complete, the ticks usually dropped from the animal but in some instances were removed by forceps. To liberate the mouse the plug of

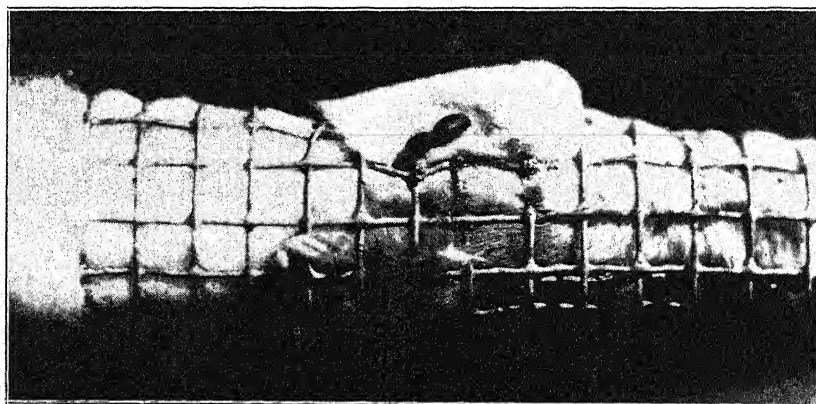


FIG. 2. Adult *Ornithodoros hermsi* feeding on white mouse confined in wire feeding cylinder.

cotton in front was removed and the animal allowed to crawl out. This method of feeding proved quite satisfactory and allowed for easy handling and observation of the ticks under a binocular dissecting scope.

The majority of the ticks attached immediately, the period of time required for complete engorgement varied with the different instars. The feeding periods for a series of several hundred ticks in all active stages are shown in Table 1.

TABLE 1

1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Did	7	11	12	10 Minimum
not	40	131	66	70 Maximum
feed	16	31	32	28 Average

OVIPOSITION

The act of oviposition, which is similar to that already observed in other species, was observed under the binocular in one instance, the adult female tick lying with the ventral surface exposed to view. The inflated, translucent, extremely thin-walled, eversible gland, Gene's organ (Samson, 1909), was noticed protruding posteriorly to and at the base of the ventral margin of the hood, between the hood and the basis capituli. This organ consists in part of two long, separable finger-like processes which, when extended, curve upward and extend over the mouth-parts, the rounded tips reaching posteriorly nearly to the genital orifice.

At this moment two eggs were observed clinging to this organ, being held in place by a viscous substance. The genital orifice was raised and drawn towards the mouth-parts by the contraction of the muscles in the region of the first pair of coxae. A third egg was observed to emerge suddenly from the genital opening; at the same time the "fingers" of the organ were extended to grasp the egg which became fastened to the other two eggs already adhering to the structure. The mouth-parts were flexed toward the genital opening in such a manner that when the organ was returned to position the mouth-parts assisted in pushing the eggs over the capitulum and hood to beyond the outer anterior margin of the body where they were added to the mass of eggs previously extruded, the egg mass being held together by the viscous material apparently secreted by the organ.

Shortly preceding the extrusion of the egg from the genital opening and continuing for a period of two minutes, a series of pulsating movements was noticed to occur under the body wall around the genital aperture. These movements continued until the organ was returned to its normal position within the body cavity. The "fingers" of the organ itself remained in an extended position during the period of egg deposition. At the end of egg deposition or when disturbed the organ slowly contracted or appeared to become deflated as the pressure of the fluid within was reduced and the fluid had receded into the body of the tick. At the same time the mouth-parts gradually assumed their normal position. The only indication as to the presence of the organ after being withdrawn into the body was the smear of the viscous material at the point where the organ entered the body wall. The time re-

quired for the deposition of a single egg from the time of its expulsion from the genital orifice until the egg was carried by the mouth-parts to beyond the margins of the body was nearly two minutes. This tick ceased oviposition due to the disturbance caused by the light of an electric desk lamp but resumed oviposition after the cover of the pill box container had been replaced as evidenced by the number of eggs which had appeared by the following day.

LIFE HISTORY STUDIES

Life history studies were conducted in the laboratory, and although the conditions best suited for the development of the various stages of ticks in the laboratory are to some extent unlike the conditions found in nature, the data obtained will give some idea as to what may be expected under field conditions.

A total of 168 larvae were used in this study representing the progeny of three female ticks, only the first batch of larvae produced by each female tick being used.

Incubation and Development of Ova

Oviposition by one female began April 8 and continued for a period of three days. At the end of this period the female tick was removed from the pill box and placed in a clean container for further observation.

The eggs were deposited in a loosely agglutinated mass easily separated by means of a dentist's probe and were of a light amber color when first deposited, darkening gradually in a few days. By the end of the 12th day the eggs darkened perceptibly on the upper side and the embryos were visible through the shell. On the 14th day the outlines of the six legs were visible and through the under surface of the eggs the outlines of the form of the developing larvae could be discerned. From that day until the eggs hatched (April 29—completed May 1) the outlines of the body and legs were plainly visible, the eggs lost their luster and on the 21st day the larvae emerged. The shells split in a plane at right angles to the longitudinal axes of the bodies of the larval ticks. The mouth-parts of each tick emerged first, followed by the first pair of legs. Attention was focused on the hatching of one egg. Muscular efforts of the body of the young tick split the shell further, the second and third pairs of legs were ultimately extricated and the larva crawled from the confines of the shell. The young larvae upon emergence were quite active but were unable to feed.

Eggs were produced in batches ranging from a few to 140 or more eggs per batch over a period of several months throughout most of the year under laboratory conditions. The incubation period averaged 17 (range 9–24) days. The percentage of hatch varied with the different batches of eggs produced by several female ticks. In some batches none was apparently fertile while in others produced by the same tick 95% hatched. The greatest percentage of hatch for any one batch was 98%.

Developmental Stages

A few of the larvae were selected and placed on a young white mouse. They were unable to feed. A few days later some of these larvae were observed to molt. The slide mounts of these skins revealed the presence of three pairs of legs and the larvae emerging from the skin still possessed the characteristic three pairs of legs. Evidently many of the larvae of *Ornithodoros hermsi* may undergo two molts while in the larval stage—the first molt occurring shortly after emergence. The first

larvae fed to repletion on white mice on May 10, eleven days after hatching. Other feedings occurred on May 13, 15, 16, 17, 19, 20, and 21. The average time for feeding was 15.7 (range 7 to 40) minutes.

Second Molt, First Nymphal Stage.—The first nymphs appeared on May 26, the last on June 14, averaging 14.2 days from the date of feeding as larvae until the date of molting as first stage nymphs. The resulting nymphs were placed individually on separate white mice and allowed to engorge. These ticks fed on an average of 4.2 (range 1 to 41) days after molting. The time required to reach engorgement averaged 20.6 (range 11 to 131) minutes.

Third Molt, Second Nymphal Stage.—The first of the second stage nymphs appeared June 13 and the last on July 27, an average of 14.1 days from time of feeding as first nymphs until molting as second stage nymphs. These ticks were placed on individual white mice and permitted to feed. This stage required an average of 32.3 (range 12 to 66) minutes to reach repletion after an average period of 15.9 (range 5 to 38) days from time of molting to time of feeding.

Fourth Molt, Third Nymphal Stage and Adults.—Molting began on July 4, 15 days after the ticks had fed as second stage nymphs, the average for the entire lot being 17 days. With this molt, 10 ticks reached maturity, four females and six males. Feeding of the ticks was conducted in the same manner as before, the average being 29 (range 6 to 49) days after molting. Repletion was reached in an average time of 27.7 (range 10 to 70) minutes.

Fifth Molt, Adults.—With the fifth molt the majority of the ticks reached sexual maturity. Molting began August 12 and continued over a period of nearly two months, the last tick molting on October 10. As the ticks reached maturity they were paired (male and female). Eggs from these matings appeared August 24 with one female (Lab. No. 7/5) producing a first batch of eggs totaling 58. The egg to egg cycle thus covered a period from April 8 until August 24—4 months and 16 days, although egg depositions from other female ticks occurred at intervals from August to January.

OBSERVATIONS ON THE PROCESS OF MOLTING

Molting of the first nymphal stage was noted. The nymph, having fed previously on a white mouse, began the process of molting 15 days after feeding. The color of the young tick had darkened from a bright red to a dull, purplish-red shade with a greyish appearance over all. The claws of each leg of the tick were attached to the floor of the pill box in which the tick was confined. The first pair of legs spread wide apart in front of the body, the hind pair of legs held in a position under the posterior end with the two intermediate pairs of legs in a normal position along the sides. The first movements observed consisted of an upward tugging motion within the old loosened skin in an apparent effort to withdraw the legs. This was followed by a fairly rapid alternate contraction and expansion of the body. The fluid in the digestive diverticulae seemed to flow towards the central portion of the digestive tract and through muscular force exerted by the muscles of the body and digestive tract, the fluid surged out to the ends of the diverticulae in wave-like motions. This extra pressure caused the old skin to rupture at the anterior-most point, and as the body protruded forward the old skin split gradually following the lateral body margins until it had reached a point approximating the first coxa on

either side. As the body protruded still further through this opening, the mouth-parts were withdrawn from the old skin. Next the first pair of legs were withdrawn by an upward tugging motion of the body and with every tugging effort the legs were drawn towards the body from the old skin until each was free. All the while a continuous surging of the fluids in the body cavity was observed. In a short time the second pair of legs appeared and the tick was then able to fasten its claws quite firmly into the bottom of the pill box. The third and fourth pairs of legs appeared in succession, the tick resting between each exertion. The tick then crawled from the remaining cast skin. The entire process required, in this instance, approximately four minutes.

In some instances the cast skin of the tick was carried about on the posterior portion of the body until the next feeding at which time the old skin slipped from the distended body of the tick. Occasionally the legs of the tick became entangled in some manner, and the tick was unable to extricate itself from the skin, in which cases when noticed the skin was removed by means of a fine scalpel or scissors. The color of the freshly molted tick varied to some extent but the majority were of a deep garnet color with a clean, glistening, greyish sheen over the body, the legs being nearly white in contrast.

Occasionally, if temperature and humidity conditions were not correct, the ticks, particularly those of the first nymphal stage, were imperfectly developed, the legs being very papery in appearance and not rounded out. This first nymphal period is evidently the most delicate period in the development of the tick, more so than any succeeding molt.

NOTES ON LONGEVITY

In experiments dealing with longevity the ticks were kept in pill boxes in the constant temperature chamber in the laboratory and the results noted do not therefore necessarily represent the exact behavior of the ticks in nature.

Table 2 indicates the length of life of 3 adult female ticks (2 of which were

TABLE 2

Female tick number	Date taken as adult	Number of feedings	Number of eggs deposited	Number of infections in laboratory animals	Date of death	Elapsed time between date of capture and date of death		
						Years	Months	Days
10	9-19-33	3	50	0	3-15-35	1	6	-
5	8-17-34	4	232	4	10-21-35	1	2	4
7 ¹	8-17-34	2	235	2	2-24-36	1	6	7

infective) taken as adults at Big Bear Lake, California. These ticks were permitted to feed several times during the interim from date of collection to date of death and during this period produced several infections in laboratory animals. The age, and the number of times these individual ticks had fed, previous to arrival in the laboratory is unknown.

One female tick (No. 11) was taken as an adult at Big Bear Lake, San Bernardino County, California, on August 17, 1934. This tick was kept under laboratory conditions for a period of 7 years, 3 months and 4 days. Assuming the life cycle to cover approximately $4\frac{1}{2}$ months, this tick would be over $7\frac{1}{2}$ years old from time of hatching until date of death.

TABLE 3.—Showing longevity of female ticks from the time of hatching to May 15, 1942 and their ability to withstand starvation. (Ticks reared under laboratory conditions)

Tick No.	Date hatched	Date last feeding	Elapsed time between last feeding and 5-15-42			Elapsed time between hatching and 5-15-42		
			Years	Months	Days	Years	Months	Days
1	5-20-35	11-23-37	4	5	22	6	11	25
2	6-14-35	11-29-40	2	5	16	6	11	1
3	"	5-25-38	3	11	20	6	11	1
4	"	5-23-38	3	11	22	6	11	1
5	"	11-24-37	4	5	21	6	11	1
6	"	5-26-38	3	11	19	6	11	1
7	"	11-24-37	4	5	21	6	11	1
8	"	6-1-38	3	11	15	6	11	1

That ticks are able to withstand long periods of starvation is shown in Table 3. It is interesting to note that these ticks at present appear to be in a semi-dormant condition, that is, movements are sluggish and they refuse to feed when placed on a laboratory animal. The bodies are still rounded out from their last meal and egg deposition has ceased.

LONGEVITY OF NYMPHAL STAGES¹

Third nymphal stage.—This series included three lots of third-stage nymphs—the progeny of three female ticks. The range of longevity was from one day to 108 days.

Second nymphal stage.—Records concerning the longevity of the second-stage nymphs show a range of from 25 days to 79 days from date of molting to death.

First nymphal stage.—A large series of ticks of this instar withstood periods of starvation ranging from 2 to 154 days.

LONGEVITY OF LARVAE

Two lots of larvae numbering well over one hundred lived without food from 30 days to 125 days.

EFFECTS OF THE BITE OF *Ornithodoros hermsi*

The generally severe bites of other species of argasine ticks have been described by many investigators. Nuttall et al (1908) cite instances in which the effects of the bites of *Argas persicus* were fatal to animals and to man on rare occasions. Livingstone (1858), referring to the wounds inflicted by the "tampan," *Ornithodoros moubata*, states the tick produces a tingling sensation which lasts for nearly one week. Dutton and Todd (1905) observed the bites of this species of tick to be quite painful and when allowed to bite a monkey noticed that, "Immediately after feeding a small crust of serosanguinolent fluid forms at the site of the bite. Surrounding it is a roseola about 2 mm in width. Two hours later the central clot is surrounded by 2 concentric zones, each 2 mm in width; the first colourless the second ecchymotic. Six hours later the clot has become almost black, and is placed at the apex of a slight colourless wheal, bordered by an ecchymotic zone about 1.5 mm in width." Wellman (1905-07) claims the bites of the young ticks to be more painful than those of the adults. Deaths resulting from the bites of *Ornithodoros turicata*, have been known to occur, according to Duges (Nuttall et al, 1908). *Ornithodoros talaje* also produces severe itching and pain by its bites (Guerin-Meneville, 1849). Herms (1916, 1939) describes the severe effects produced by the bite of *Ornithodoros*

¹ Longevity records of larval and nymphal stages represent periods of starvation from date of molting to date of death.

coriaceus and states that the natives in certain parts of California fear the bites of this tick more than the wound inflicted by a rattlesnake. Lesions produced on the skin of the arm of man "showed a small dark purple ring surrounding a bright red spot, the point of attachment. The discoloration disappeared in a short time, but the arm was 'highly irritable for two or three days and at the point of attachment a minute clear scab formed.'" Nuttall et al (1908) state, "their bites were intolerably sharp and painful, and wounds bled a good deal—but notwithstanding, there has been intermittent irritation ever since."

The bites of *Ornithodoros hermsi* appear to be quite benign in that persons contracting the disease, relapsing fever, seldom report having been bitten by any tick or insect (Porter et al, 1932). Larval ticks placed upon the arm of the writer and an assistant, engorged to repletion and detached in approximately fifteen minutes with no evidence of pain either at the time of the insertion of the ticks' mouth parts or at any time during the period of feeding. At the point of entry of the mouthparts a small hemorrhagic area appeared which was scarcely visible and would have been overlooked had we not known that the ticks had attached. Larval ticks allowed to feed upon white mice produced a more severe reaction in most instances. The wound itself evidently was not painful to these animals although the hemorrhagic area on the skin was larger, measuring on an average a little over 1 mm in diameter, with a bloody exudate usually forming in the center. Occasionally this area became swollen and tinged a dark purplish color resembling the type of wound inflicted by the Pajaroello tick, *Ornithodoros coriaceus*. The lesions produced by the nymphal ticks are also apparently quite benign and cause no evident discomfort to the experimental animal. In the majority of the wounds inflicted by adult ticks on experimental monkeys a bright hemorrhagic area appeared approximately five minutes after the mouth-parts were inserted into the skin of the animals. These areas averaged 3 mm in diameter forming a rather serrate-edged wheal. In the center of each area a blood-tinged exudate appeared as soon as the tick had detached. For a few days following the feeding of six adult ticks on a monkey, the animal was noticed to scratch vigorously at the wounds caused by the ticks. Results of experiments in which infective ticks were allowed to engorge on blood of humans demonstrated that the bite of the tick, *Ornithodoros hermsi*, produces little discomfort at the time of feeding. With but few exceptions no sensation was experienced by these volunteers during the time the ticks were attached. A slight stinging sensation was noted by two of the men at the time the ticks inserted their mouth-parts into the skin (Wheeler, 1938).

SUMMARY

Observations relating to the field biology of *Ornithodoros hermsi*, Wheeler, Herms and Meyer were based on notes taken by the author while in the field and supplemented in part by records from other investigators. Notes concerning life history studies were recorded as the ticks developed under laboratory conditions and therefore do not necessarily represent the true picture of the requirements for development in nature. From both field and laboratory observations:²

1. *Ornithodoros hermsi* occurs in the western United States at elevations above 3000 feet, with records of capture from California, Idaho, and Colorado.

² Observations on life history studies are represented by the author's own findings which may differ to some extent from those of other investigators.

2. This species of tick is apparently a parasite of small mammals, particularly of species of western chipmunks belonging to the genus *Eutamias*.

3. The act of oviposition is similar to that observed in other tick species. Eggs are produced in batches ranging from a few to 140 or more per batch. The greatest number of batches for any one female tick was four with a total of 232 eggs.

4. The incubation period of the eggs was 17 (range 9–24) days, while the percentage of hatch ranged from 0 to 98%. Ticks were reared in a constant temperature and humidity cabinet with optimum temperature and humidity of 75° F and 90 per cent respectively.

5. Development from egg to egg covered a period of four months and 16 days requiring five instars from egg to adult with many of the larvae passing through two instars. The process of molting is described.

6. Feeding of the ticks in various instars was accomplished by allowing the ticks to engorge on the blood of white mice immobilized in a cylindrical wire-cloth cage. The range of the average feeding times for the different instars was from 16 to 28 minutes.

7. Longevity of laboratory-reared ticks, with occasional feedings, was noted to cover a period of over six and one-half years from date of hatching to date of death. Infective adult ticks of unknown age taken in nature were observed to live in the laboratory for over one and a half years.

8. Female ticks were shown to be able to withstand starvation for over four years.

9. As compared to other species of *Ornithodoros*, the bite of *Ornithodoros hermsi* is quite benign as shown by experiments in which ticks were allowed to feed upon laboratory animals and human volunteers.

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CHARACTERISTICS OF THE POPULATION AVAILABLE FOR
BIOASSAY OF ANTHELMINTICS IN *NIPPOSTRONGYLUS*
MURIS INFECTION IN ALBINO RATS¹

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The growing need for a more accurate method of assaying anthelmintics has suggested the use of *Nippostrongylus muris* infection in standard albino rats as a source of test material. Although it has been used in many of the recent quantitative experiments in immunity and resistance and once in analyzing anthelmintics (Schwartz and Porter, 1937), no experiments designed to discover how the worms varied from one host to another have been reported. Since such knowledge is a prerequisite to accurate statistical analysis of the proposed type of anthelmintic tests, the present experiments are reported to fill this lacuna and to suggest methods of stabilizing the technic.

MATERIALS AND METHODS

Albino rats from a commercial source or from our own stock, which is closely related, were used in these experiments. One group (#9) was made up of females; the rest were all males.

Dr. Asa C. Chandler of Rice Institute kindly supplied us with a culture of *N. muris* which he had used for a number of years and suggested methods for culturing the extra-host forms of the nema. These consisted of mixing fecal matter with helminthologically sterile animal charcoal and dirt, adding sufficient water to form a thick paste. This was placed on fluted filter paper in a Petri dish, leaving about one-quarter to one-half inch of the paper free of the culture. As the infective larvae develop they migrate to the free edges of the filter paper, from which they may be recovered by placing the feces-free pieces of filter paper in a tea strainer in a finger bowl full of 0.5 per cent sodium chloride solution. After standing for approximately one hour, the nemas were concentrated by centrifuging and draining the supernatant fluid so that 0.3 to 0.5 ml contained approximately 500 worms. Whenever samples for injection or counting were drawn from the suspension, it was always thoroughly agitated. In addition the suspension within the inoculating syringe was kept agitated with a small air bubble.

The actual number of larvae in the concentrated suspension was estimated by counting the worms in five 0.02 ml samples from a single tuberculin syringe, and the amount of suspension necessary to infect each rat with approximately 500 worms was injected subcutaneously into the abdomen immediately medial to the anterior portion of the right fold of the flank. Since Chandler (1935) has shown that immune rats often react to subsequent injection by the formation of abscesses, this technic prevented the accidental use of immune animals.

The rats were killed on the 10th day with ether and the small intestine removed.

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The contents were first gently squeezed out with the fingers or a five-inch hemostat, then more vigorously with a round instrument and a glass plate. From time to time the stripped intestines were examined for worms, but none were ever found.

The intestinal contents were mixed with sufficient 5 per cent NaHCO_3 solution to fill a small (50 ml) bottle and allowed to stand in an ice-box for at least 24 hours. The bicarbonate solution cleared and dissolved the mucus and mucosa and unless the animal had access to food in the 24 hours prior to death, the only solid opaque particles left after the refrigeration period were the reddish worms.

The worms were counted by the following technic.

1. The supernatant fluid was poured into a filter paper in a funnel.
2. The remaining fluid containing worms and debris was vigorously shaken and poured into one or more cup-shaped filter papers on an absorbent pad.
3. The bottle was washed and the washings placed in the filter paper of step 1.
4. When the excess fluid was removed from the filter papers the worms were counted.

EXPERIMENTAL RESULTS

A. The Worms Injected

The characteristics of the worm suspension used for infecting the rats have been examined. To obtain material for statistical analysis five 0.02-ml samples were taken from each of 26 tuberculin syringefuls of a single suspension of infective larvae and counted, observing the usual precautions to keep the suspension uniform.

The sampling of the suspension of larvae was examined first by the analysis of variance in Table 1. The variation between syringefuls did not exceed the combined error between the five 0.02-ml samples from each of the 26 loadings to a significant degree, which indicated that the successive syringefuls were almost equally good samples of a uniform suspension. The variation between syringefuls in Table 1

TABLE 1.—Analysis of variance of sampling of suspension of infective larvae of *Nippostrongylus muris*

	Degrees of freedom	Sum of squares	Mean square
Between syringefuls	25	907	36.28
Within syringefuls	104	2904	27.92
Total	129	3811	29.54
F = 1.29			

included two components, the variance associated with different fillings of the syringe and that between samples within fillings. When these were separated as suggested by Snedecor (1940) the first component was equal to 1.27 as compared with the much larger 27.92 within syringefuls. Hence the accuracy of estimates of the number of worms in a suspension could be increased nearly as well by increasing the size or number of counts from each loading of the syringe as from increasing the number of syringefuls. Neglecting differences between and within syringefuls the variance for a sample of 0.02 ml from this suspension was computed from the total row of the table as 29.54 and its standard deviation as 5.43.

The relative uniformity of the separate syringefuls suggests that the successive 0.02-ml samples from a single loading may have agreed with one another within the sampling error. In this case they should be distributed in the Poisson series, which

has the characteristic that its variance is equal to the mean. In the present case, the variance within syringe-fuls of 27.92 was considerably larger than the mean of 16.29 worms per 0.02-ml sample. Moreover the chi-square test for homogeneity of the 0.02-ml samples with their respective means for the separate charges of the syringe, computed as described by Fisher (1936), gave $\chi^2 = 174.39$ with $n = 104$. Since the variability exceeds that for random samples very significantly, it is clear that even the present precautions did not insure a uniform discharge of the larvae within the syringe in the five 0.02-ml samples. In practice approximately 500 worms or more than 30 such samples were injected at one time into the rats. For this reason, the lack of uniformity in the rate at which the larvae were discharged in small samples was smoothed out so that the variation in the number injected probably approached a more symmetrical and normal form. Yet the variability between loadings was even a little larger than that within syringe-fuls, emphasizing the need of testing the variability experimentally rather than estimating it on the basis of the Poisson series.

Keeping this limitation in mind, the combined variance obtained with the 0.02-ml samples ($s^2 = 29.54$) has been used to compute the error expected for a 0.5-ml sample. Since 0.5 ml contains twenty-five 0.02 ml, the observed variance for 0.02 ml was multiplied by 25 to obtain the variance of the total or $25 \times 29.54 = 738.5$, from which the standard deviation was estimated as the $\sqrt{738.5} = 27.18$ or 6.7 per cent of the 407 worms expected in a sample of this size. If only the errors of random sampling had been involved, the coefficient of variation would have been 5.0 per cent instead. This indicates that for accurate estimate of the number of worms in a suspension, the total volume examined should equal or exceed the quantity injected per rat. For the standard "dose" of 500 larvae in 0.5 ml, the error would then fall between 5 and 10 per cent of the estimate. Moreover, it would be preferable to sample several rather than one syringe-ful.

B. The Worms Established

Table 2 contains the pertinent information as to the age of rat, inoculating dose, numbers of worms establishing themselves, etc. Some of these rats had been treated by apparently ineffective anthelmintics. Analysis of variance of the number of worms established revealed a highly significant variation between tests. These variations were not due to any important difference between the variances of the separate groups, which when tested by chi-square did not differ significantly ($\chi^2 = 11.9$, $df = 10$).

The number of worms in the infected rats was subject to two unequal sources of variation; a large source between different groups of experimental animals and a smaller one within each sample group. In the absence of any seasonal or other type of curve to correct the secular variation, the ratio of the standard deviations in Table 2 provided the most satisfactory basis for adjusting the individual observations. Following the example provided by Bliss and Hansen (1939) a constant factor was added to each term in a group which would reduce the departure of the group mean from the general mean to $\frac{48.88}{248.80} = 0.1964$ of the original observed difference. This adjusted the individual values for secular variation so that the standard deviation based upon all 110 of the adjusted numbers of worms establishing themselves was the same as that observed originally within each sample population.

TABLE 2.—Data and statistics of numbers of *Nippostrongylus muris* reaching maturity in groups of rats receiving the same dose of infective larvae

Test number	1	2	3	4	5	6	7	8	9	10	11
Date of death of host	8/31/40	5/10/40	11/23/40	11/9/40	10/27/40	9/14/40	12/7/40	1/18/41	1/25/41	2/9/41	3/9/41
Age (days) of rats at time of infection	50	53	43	60	55	52	50	60	67	...	55
Av. wt. of rats at time of infection (grams)	140	140	125	134	135	132	111	136	112	149	127
Av. wt. of rats when killed (grams)	144	132	137	135	110	146	131	139	118
No. of larvae injected	520 ± 40	567 ± 70	505 ± 50	503 ± 40	504 ± 52	654 ± 153	493 ± 22	500 (?)	508 ± 17	508 ± 35	499 ± 60
No. of worms established (X)	113 131 121 204 212 199 177 122	362 323 357 314 352 287 298 278 289 371	219 198 197 176 286 228 246 207 185 242 131	292 325 303 258 243 246 261 369 288 273 308	314 178 315 321 295 287 294 226 226 240	129 159 130 67 129 123 142 108 102 100	349 417 299 263 244 295 312 326 362 310	338 311 274 303 279 334 198 229 369 328 310	378 275 412 265 286 421 462 400 378 413	172 335 335 282 250 270 252 278 311 182 181 295	318 471 346 381 340
Mean (\bar{X})	159.9	328.1	210.4	296.0	269.6	117.0	317.7	297.5	369.0	261.9	371.2
Variance (V)	1,781	1,375	1,684	2,521	2,365	634	2,472	2,487	4,756	3,288	3,623
Standard deviation (s)	42.2	37.1	41.0	50.2	48.6	25.2	49.7	49.9	69.0	57.3	60.2
Coefficient of variation	26.4	11.3	19.5	16.9	18.0	21.5	15.6	16.8	18.7	21.9	16.2

Analysis of Variance of Data

	df	Sum of Squares	Mean Square	Standard Deviation
Between tests	10	619,051	61,905	248.80
Within test groups	99	236,480	2,389	48.88
Total	109	855,531		
			F = 25.9	

Since some of these corrections were positive and some negative, the general average number of worms establishing themselves in these rats remains the same. The resulting distribution was tested for both skewness and kurtosis (Snedecor, 1940). Neither g_1 measuring skewness nor g_2 measuring kurtosis exceeded its standard error ($g_1 = 0.132 \pm 0.230$ and $g_2 = 0.348 \pm 0.457$) indicating that the distribution was symmetrical and normal in form.

This distribution, however, was not binomial. Using the average number of worms established in these experiments from 500 infective larvae injected, a mean of 269 gave $p = 0.538$ and $q = 0.462$. According to the binomial theorem the variance pqn would be expected to equal $0.538 \times 0.462 \times 500 = 124.3$. The observed variance $\frac{S(x-\bar{x})^2}{n} = 2389$ or nearly twenty times the binomial value.

DISCUSSION

From the foregoing data it appears that the variation in numbers of *N. muris* infective larvae injected into rats under the conditions described was relatively small and probably normally distributed. Although the number of infective larvae in small-sized samples of the suspension varied rather widely, this irregularity was proportionately much smaller in the large samples injected into experimental rats under the conditions described.

However, an extremely large secular variation appeared in the number of adult parasites establishing themselves. This phenomenon has been noted by Chandler (1935) who was unable to find its cause. A part of it was undoubtedly due to the inaccurate estimate of the number of worms injected provided by the 0.02-ml samples.

The within-sample variation in numbers of worms established resembled that of the numbers of worms injected in that both were symmetrical and approached the normal curve in form. However, the former variation was relatively more extensive as measured by the larger coefficient of variation. This larger variation in numbers of worms established than in the numbers of worms injected predicates some action detrimental to the parasite on the part of the hosts used in these experiments, none of which were immune or age resistant.

Since the within-sample variation in *N. muris* infection was uniform and symmetrical even though large, it should be possible to determine with sufficient accuracy the numbers of these parasites killed by the lethal action of any drug from parallel counts of worms in treated and untreated infected rats. This fact has been used successfully to develop a new method of anthelmintic bioassay (Whitlock and Bliss, 1943) which is more accurate and less tedious than a Hall critical test with the present type of parasitism.

The use of a parasitic infection whose characteristics are known and can be controlled should be of as much assistance to the chemotherapy of enterohelminthiasis as other standard infections are to the chemotherapy of syphilis, malaria, and various bacterial diseases.

SUMMARY

1. A standard technic for inoculating rats with uniform doses of *Nippostrongylus muris* is described and its error determined.
2. Analysis of data from 110 rats divided into eleven groups and infected with

uniform doses of infective larvae of *N. muris* revealed two types of variation in numbers of worms establishing themselves: a. A within-test variation which was larger than the binomial but uniform and approximating the normal curve; b. A between-test variation which was many times larger than the above and which was unpredictable.

3. The results of these experiments show that under adequate statistical and biological control it is possible to predict with workable accuracy the number of worms in rats of a treated group expected in the absence of treatment from the number of worms in a parallel control group.

4. Although part of the variation between and within separate tests was due to variation in the numbers of infective larvae injected into the rats, this did not explain the wide variation observed. More probably it was largely due to an interaction between host and parasite which has yet to be explained.

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A BIOASSAY TECHNIQUE FOR ANTHELMINTICS¹

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The present paper describes a quantitative technique for the biological assay of anthelmintics in vivo which depends upon the mortality of a nematode in albino rats following graded doses of drug. Since small dead worms cannot be counted readily in fecal matter, the mortality of the parasite has been estimated from the numbers surviving in the treated animal and in parallel untreated controls. The practicability of basing mortalities upon the infection in the untreated host has been demonstrated by the study (8) of the numbers of *Nippostrongylus muris* which mature after the subcutaneous injection of approximately 500 infective larvae. Although the number varied considerably from one rat to another, the variation approached the normal curve in form. The limits were narrow enough to permit estimation of the mean number exposed to the drug in an experimental group of rats from the mean number of worms in a control group infected at the same time.

The percentage mortality of the parasite within the host has been related to the dose of drug so as to examine the frequency distribution of the just-toxic-dose. Earlier investigations on the toxicity of poisons to nemas in vitro (7, 9) have demonstrated the applicability of the normal distribution to the length of survival. If similar relations could be shown in vivo, the anthelmintic action of the drug could be defined more precisely than heretofore. By substituting exact definitions for the "minimal lethal dose" and similar concepts, it should be possible to replace terms such as

$$\text{chemotherapeutic index} = \frac{\text{minimal lethal or maximal tolerated dose}}{\text{minimal effective dose}}$$

with more reliable measures of therapeutic effect that are commensurate with the recent work in quantitative pharmacology. Current statistical procedures have been adapted to the present data as hereinafter described.

MATERIAL AND METHODS

The albino rats in these experiments were obtained from a single commercial source or from a closely related stock. At the time of testing they varied in age from 55 to 96 days, although within any one test group the range was three days or less. Using a technique that has been described elsewhere (8), they were infected with *Nippostrongylus muris* from a stock culture supplied originally by Dr. Asa Chandler of Rice Institute. Briefly, extra-host forms of the parasite from charcoal and earth cultures were suspended in a 0.5 per cent solution of sodium chloride and the number of larvae in suspension determined by a count of several random samples. An amount of suspension sufficient to infect each rat with a predetermined number of worms, usually 500, was then injected into the subcutis of the abdomen immediately medial to the anterior portion of the right fold of the flank. On the 8th day after

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the infection, the rats were treated with anthelmintic and on the 10th day killed and the worms recovered and counted.

As a result of preliminary experiments, rats were assigned at the time of treatment to the five or six groups in a test so as to balance body weight, except for those in Test III of the first experiment. Random assortment to groups, as used in Test III, perhaps, would have given equally good results. The balancing of body weights was intended to correct any possible bias in dosage, since all rats in a group received the same amount of drug. Within the weight range in these experiments (110–160 grams), however, no inverse correlation between efficiency and body weight has been noted.

The experiments fell into three main series and in all three series carbon tetrachloride served as the drug. The required dose was dissolved in mineral oil and administered through a metal stomach tube with a tuberculin syringe. In all cases the chemicals conformed to U. S. P. standards.

The first experiment was the most extensive. It measured the mortality of the parasite in the host at 4 to 5 dosage levels in five different tests. In Test I the four doses were varying amounts of a 10 per cent solution of carbon tetrachloride, but in the remaining series the dosages of drug were dissolved uniformly in sufficient mineral oil to make 0.5 cc.

The second experiment determined the dosage-mortality curve of carbon tetrachloride for albino rats. Although the rats were from the same source as those used in the first experiment, they were not infected with nematodes prior to treatment. As a second difference in procedure, the drug was administered to the rats in undiluted form without mineral oil or other purgative.

The third experiment tested the effect of increased "doses" of infective nematode larvae upon the efficacy of carbon tetrachloride. The standard inoculation of 500 larvae was doubled and doubled again to establish higher populations of parasites in the host. Eight days later each rat received a therapeutic dose of 0.26 cc of drug dissolved in sufficient mineral oil to make 0.5 cc and on the 10th day the surviving worms were counted in postmortem.

Statistical techniques are available (3) for the evaluation of dosage-mortality data, where both fatalities and survivors can be counted directly. Here, however, only the surviving nemas could be observed. The number of potential survivors killed by the anthelmintic had to be estimated by difference from the mean number of worms in the untreated control animals. Because the individual rats varied widely in their response, the usual computation has been modified, as will be noted later.

RESULTS OF EXPERIMENT I

The toxicity of carbon tetrachloride in solutions of mineral oil was determined in five tests involving 129 rats infected experimentally with *Nippostrongylus muris*. Table 1 shows for each rat the dose of drug administered and the number of worms recovered. The purpose of the experiment was to determine the relation between dosage of carbon tetrachloride and percentage mortality of the nematode. It was assumed in the computation that without drug treatment, each rat would have been infected with the same number of worms as the mean of the corresponding group of controls. The difference ($\times 100$) between the number expected and the number observed, divided by the number expected, gave the percentage of nemas in each rat killed by the drug.

TABLE 1.—*Number of adult Nippostrongylus muris recovered from albino rats ten days after injection with 500 larvae and two days after treatment with varying doses of carbon tetrachloride, with the mean and standard deviation for each group*

Test No.	Number of worms postmortem with following dose per rat of carbon tetrachloride					
I	Control	0.016 cc	0.032 cc	0.063 cc	0.126 cc	
	279 338 334 198 303 290.4 57.0	328 311 369 336.0 29.8	229 274 310 271.0 40.6	210 285 117 204.0 84.2	63 126 70 86.3 34.5	
II	Control	0.032 cc	0.063 cc	0.126 cc	0.251 cc	
	378 275 412 265 286 323.2 67.0	421 462 400 378 413 414.8 31.0	207 17 412 74 116 165.2 154.4	123 143 192 40 259 151.4 81.4	0 0 0 25 0 5.0 11.2	
III	Control	0.032 cc	0.063 cc	0.126 cc	0.261 cc	
	172 335 335 282 250 270 274.0 60.8	252 278 311 182 181 295 249.8 56.4	273 211 294 291 231 241 298 262.7 34.8	194 7 222 107 161 15 117.7 91.1	10 2 0 2 1 0 2.5 3.8	
IV	Control	0.08 cc	0.12 cc	0.14 cc	0.20 cc	0.26 cc
	381 346 340 471 318 371.2 60.2	349 269 314 61 468 292.2 148.8	309 41 372 235 299 251.2 127.1	180 140 22 1 275 123.6 113.7	3 0 84 21 27.0 39.1	1 1 0 0 0.5 .58
V	Control	0.10 cc	0.12 cc	0.16 cc	0.20 cc	0.26 cc
	297 274 300 213 199 256.6 47.5	128 51 176 219 143.5 72.0	317 5 65 55 116 111.6 121.4	5 172 132 186 11 101.2 87.4	3 2 0 4 5 2.8 1.9	0 3 0 0 0 0.75 1.5

The percentage mortalities were subject to variation (1) in the number of worms expected in the absence of the drug, and (2) in the susceptibility of the host to drug treatment. In an earlier study (8) with the same type of infection but without an anthelmintic, the number of worms recovered from each rat varied more than would be expected by chance. This has been checked in the present experiment. If the control rats in a given test had been equally subject to infection by nemas, the

TABLE 2.—*Analysis of the variance in the number of nemas which established themselves in the untreated control rats*

Variation	Degrees of freedom	Mean square	Variance ratio
Between tests	4	10468	3.011
Within tests	21	3477	1
Expected from binomial distribution		116	

variance observed between rats should approximate the theoretical sampling variance expected from the binomial distribution. The observed variances, however, were 18 to 39 times as large as those expected for equal exposure and equal susceptibility to infection, giving a combined standard deviation within tests of 59 worms. Despite this wide variation between individual rats, the average number of worms varied significantly between the five tests. Hence the number expected in each treated rat if the anthelmintic had been withheld could be estimated more accurately from the mean of the controls in the same test than from their general mean (302 nemas) over all five tests.

The above source of heterogeneity, however, accounted for only part of the variation between the rats in the treated groups. Both the observed variance and that expected from the binomial distribution were computed for all treated groups, omitting those where either the number of worms averaged more than in the controls or where one or more rats in the group were freed of them completely. The ratio of the observed variance to that expected by chance averaged 162.7 among treated groups as compared with 30.4 in the untreated controls. This five-fold increase in the variance was due, presumably, not only to the use of an "expected" instead of an observed value for the number subject to removal by treatment, but also to a significantly better response to the drug in some rats than in others.

THE DOSAGE-MORTALITY CURVE FOR *Nippostrongylus muris* IN VIVO

With many species of multicellular organisms, percentage mortality may be plotted against the dosage of drug as a sigmoid curve and the same relation would be expected here. Using methods described by Gaddum (6) and by Bliss (1), these curves can be transformed to straight lines and computed. With the equations for such lines one can determine the kill expected from a given dose of drug, or, conversely, the dose required to obtain a given mortality. Percentages are transformed by a table based upon the normal curve of error to units called "probits" (1), while doses are usually changed to logarithms. In the present case, however, the probits for the data in Table 1 could be plotted against dose directly as a single straight line rather than against its logarithm (Fig. 1).

For a precise determination of the dosage-mortality curve, the computation is made from "corrected" probits and weights (3). While corrected probits are essential for the observations at 0 and 100 per cent kill, they are also applicable to the intermediate mortalities. In terms of corrected probits, the mortality of the worms within groups of rats varied considerably less than in units of the empirical probits which are read directly from tables such as that given by Fisher and Yates (4). Hence, they have been used here in all computations and figures. In neither unit, however, was the variance related to the mortality in the theoretical form upon which the usual weighting coefficients are based. The variance within groups seemed to be either independent of the expected mortality or reduced at the larger doses. In consequence, the probit mortality determined from the worms in each rat has been given unit weight in computing the curves in Figs. 1 and 2 and in the analysis of variance in Table 3. The mortality from 0.063 cc of carbon tetrachloride in the second test averaged so much higher than would be expected from the remaining data as to suggest the presence of some extraneous factor. This mean has been identified by a shaded circle in the figures and omitted from the computations.

In contrast with the unstable mean susceptibility in many toxicological experiments, the individual dosage-mortality curves for the five tests of the present series agreed with each other well within the experimental error. The variation between

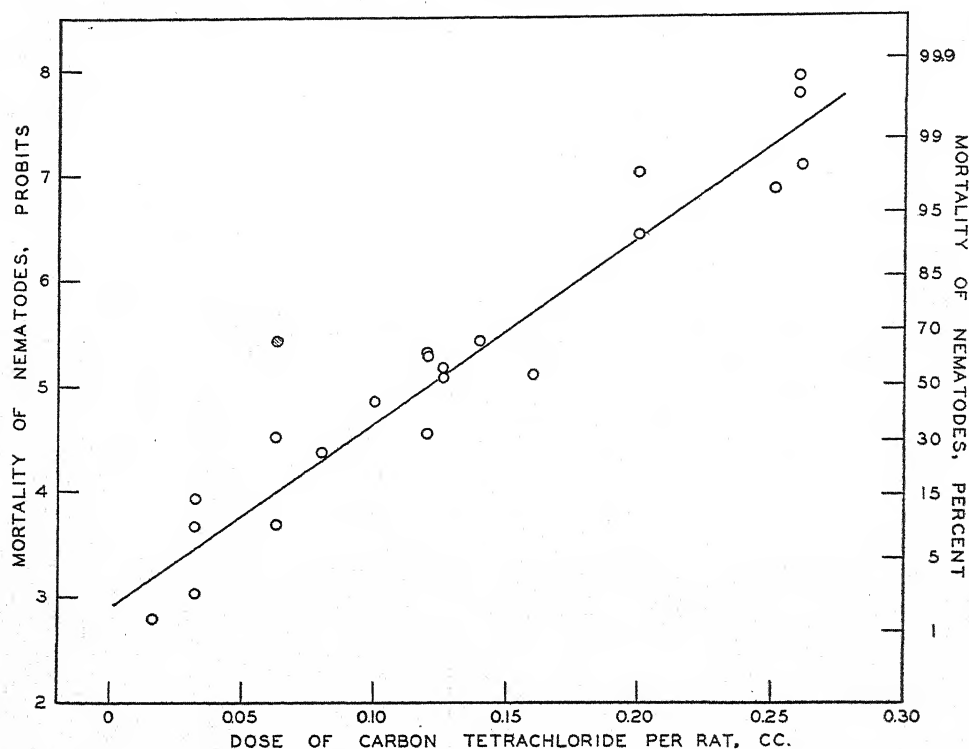


FIG. 1. Dosage-mortality curve for toxicity of carbon tetrachloride to *N. muris* in albino rats in terms of probits and dose, each circle representing the mean corrected probit for the kill of nemas in a group of three to seven rats. Shaded circle omitted in computing the curve.

the individual curves, both in position and in slope, has been compared in the analysis of variance in Table 3 with the scatter of the group means about the separate curves for each test and with the differences between rats within groups. It is evident

TABLE 3.—Analysis of the variance between equally-weighted probits as a linear function of the dose of drug, omitting the observations at 0.063 cc in the second test

Variation due to	D. F.	Mean square
Slope of single curve for all tests	1	181.421
Differences between curves in position	4	0.570
Differences between curves in slope	4	0.632
Scatter of group means about the curves for the five tests	11	0.748
Differences between rats within groups	79	0.997

from the analysis that the curves for the five tests did not differ from one another. The toxicity of carbon tetrachloride to *Nippostrongylus muris* in albino rats, therefore, seems to fall in the small group of cases having a dosage-mortality curve that is stable both in slope and in position.

The five tests in Table 1 could be represented by the single dosage-mortality curve plotted in Fig. 1 from the equation

$$y = 5.237 [\pm 0.098] + 17.46 [\pm 1.25] (x - 0.1334) = 2.909 + 17.46x,$$

where y is the mortality in probits and x the dose of drug in cc, and the square brackets [] enclose the standard errors for position and slope. According to this equation, no drug at all should kill 2.909 probits or 1.8 per cent of the worms. Yet more nematodes survived the smallest doses of drug in the first two tests than in the corresponding controls, which points to a threshold dose below which the drug is inactive or may even favor survival of the nematodes. Hence the dosage-mortality curve is not suitable for extrapolation to dosages below approximately 0.032 cc of drug, a range which fortunately is not of practical interest. The curve is exceptional in that probits have been plotted against the dose directly instead of against the logarithm of the dose so widespread in other toxicological studies.

The mortalities in probits were then replotted against the logarithm of the dose.

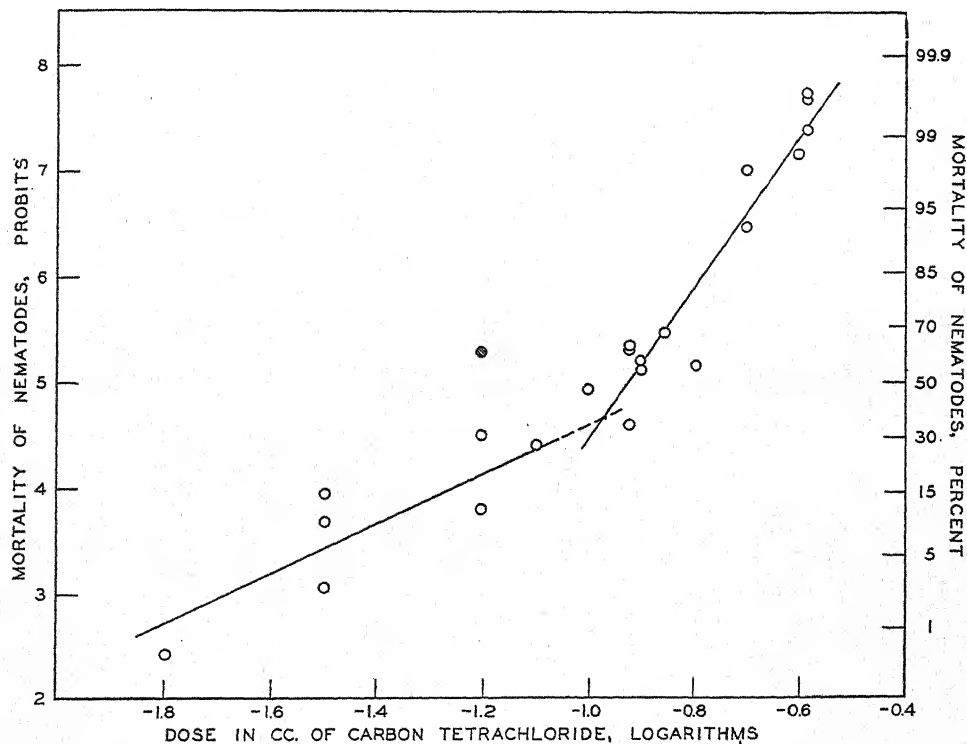


FIG. 2. Same basic data as Fig. 1 but with nema mortality plotted against the logarithm of the dose. Shaded value omitted in computing the curve.

At doses corresponding to 0.10 cc or more, the observations could be fitted by one straight line and at smaller dosages with a second straight line of lesser slope (Fig. 2). A change of slope in this direction at the lower dosages has been observed not infrequently in dosage-mortality curves, although it rarely disappears upon changing to an arithmetic scale as here. Some discontinuity between mortality and log-dose might be anticipated, indeed, from the heterogeneity between rats. A change in slope does not preclude computing a dosage-mortality curve that is valid for the upper range of mortalities. Since this is the zone of practical interest to the parasitologist so far as the parasites are concerned, the part of the curve below the "break" may be neglected. The equation of the curve plotted to the higher mortalities in Fig. 2 was

$$y = 5.999 [\pm 0.099] + 7.144 [\pm 0.683] (x + 0.7817) = 11.583 + 7.144x,$$

where y is the mortality in probits, x is the logarithm of cc of carbon tetrachloride, and the square brackets [] enclose the standard errors of position and slope.

For interpolation of the dose required for a given kill, the dosage-mortality curve in either Fig. 1 or 2 should indicate approximately the same value. For 99 per cent kill, for example, the expected dose from the curve in Fig. 1 was 0.253 ± 0.010 cc and that from the curve in Fig. 2, 0.254 ± 0.013 cc. Extrapolating, the dose expected to kill all but one worm in 500 as estimated from the first curve was 0.285 ± 0.012 cc and that from the second curve, 0.303 ± 0.020 cc. Hence, the logarithmic curve was the more conservative for extrapolation, even though not significantly different at 99.8 per cent kill from that based directly upon the cc of drug.

AN ALTERNATE TEST OF THE TOXICITY TO NEMATODES

Instead of expressing the effectiveness of the drug in terms of the percentage kill of nematodes within rats, one could measure the percentage of rats freed completely of nematodes at each dose. The first technique is the more efficient since a separate estimate of the mortality is available from each rat, while by the second procedure each rat is classified only as positive or negative. Moreover, even without an anthelmintic, the rats would eliminate most if not all of the nematodes if the examination were postponed for 10 days or two weeks. Hence, any apparent practical advantage in using the percentage of rats without nematodes is illusory.

For comparison with the former, preferred, technique, the percentage of rats freed completely of worms in each group of Table 1 has been changed to probits and plotted against the log-dose. Computed with corrected probits and weights, the equation for the dosage-mortality curve was determined as

$$y = 4.655 [\pm 0.231] + 9.79 [\pm 2.73] (x + 0.645) = 10.964 + 9.79x,$$

where y is the probit for the per cent of rats without worms, x is the log-dose of drug in cc per rat, and the square brackets [] enclose the standard errors for position and for slope. From this equation the dose which would be expected to eliminate the parasites completely from 99 per cent of the rats is the antilogarithm of $1.628 \pm .080$ or 0.425 cc, and from 99.8 per cent of the rats 1.685 ± 0.095 or 0.484 cc. In comparison with the percentage kill of nemas within rats, the doses were 2.4 times as large with an even greater discrepancy in the error of the estimate, so that the first technique used the host material much more efficiently. Whether it tends to underestimate the therapeutic dose cannot be judged from the present experiments, since all rats were examined at the same interval (2 days) after treatment.

TOXICITY OF CARBON TETRACHLORIDE TO RATS

The first experiment (Table 1) concerned the toxicity of carbon tetrachloride to the parasite *in vivo*; it was next essential to determine its toxicity to the host. The drug was administered to albino rats at four different dosage levels with the results shown in Table 4. The percentage kills have been transformed to probits and plotted

TABLE 4.—*Toxicity of carbon tetrachloride to albino rats*

Group No.	Dose of drug cc per rat	No. of rats	
		Treated	Dead
1	0.4	7	2
2	0.5	7	2
3	0.63	7	5
4	0.8	6	5

in Fig. 3 against the log-dose. The observations agreed satisfactorily with the straight line computed with both corrected probits and weighting coefficients ($\chi^2 = 0.818$, $n = 2$). In this case, too, the data would permit calculating the dosage-mortality curve directly from the dose with nearly as good a fit as for the logarithm of the dose. So many more cases have been reported, however, where the log-dose gives a straight line and the dose does not, that a normal distribution for the underlying susceptibilities in logarithmic units seems to be the rule and in arithmetic units the exception. Hence, when the data permit either interpretation as here, it is preferable to follow the rule rather than the exception.

The equation of the curve plotted in Fig. 3 may be written as

$$y = 5.040 [\pm 0.259] + 5.76 [\pm 2.48] (x + 0.259) = 6.533 + 5.76x,$$

where y is the mortality of albino rats in probits, x is the log-dose in cc per rat and the square brackets [] enclose the standard errors for position and for slope. The convex and concave curves in Fig. 3 indicate limits of one standard error below and

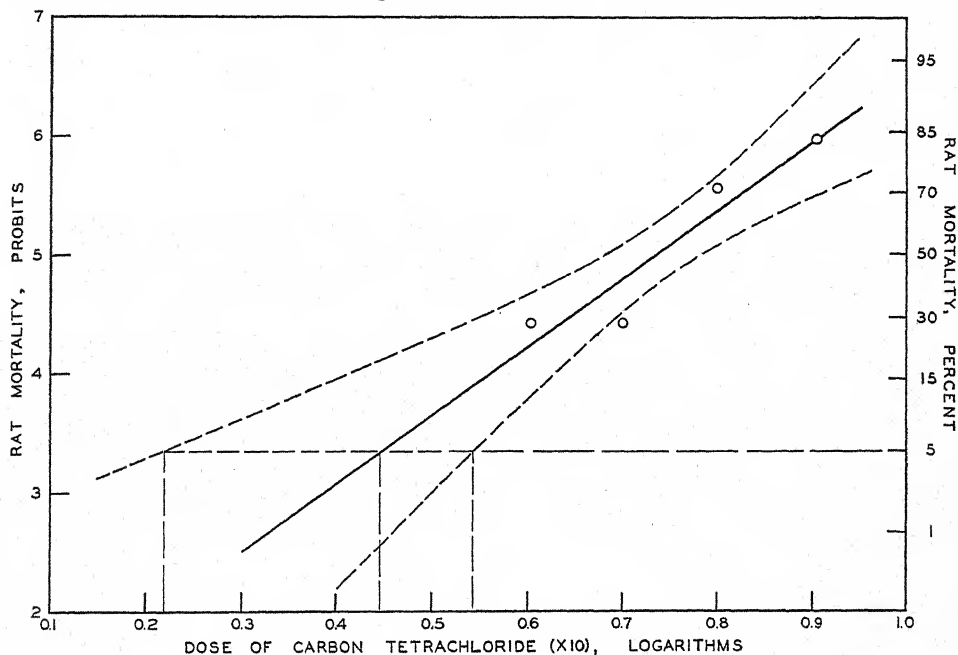


FIG. 3. Dosage-mortality curve for toxicity of carbon tetrachloride to rats. The broken lines fall 1 standard error above and below the curve. Their intersections with the ordinate for 5 per cent kill have been projected to the base to show the unequal errors above and below the dose expected to kill 5 per cent of the rats.

above the straight line. The greater the distance from the mean log-dose or mean probit, the more a point on the estimated curve is subject to the standard error of slope. Zones such as those enclosing the dosage-mortality curve in Fig. 3 apply equally to Figs. 1 and 2, but due to the larger number of determinations are there so much smaller that they have been omitted from the diagrams.

THE EVALUATION OF ANTHELMINTICS

The reliability of an anthelmintic increases with the ratio or difference between the therapeutic and the toxic dose. The so-called chemotherapeutic index is a loosely-

defined attempt to estimate this factor. With more complete knowledge of the relation between dose of drug and the biolytic reaction of the nematode and between dose and toxic symptoms in the infected host, the index can be evaluated much more accurately. Foster (5) has proposed that drugs be rated by a "standard safety margin," which he bases upon the ratio between the dose killing one per cent of the experimental animals (LD1) and that curing 99 per cent of them (ED99). He defines the standard safety margin as $\left(\frac{LD1}{ED99} - 1\right) 100$. When dosage-mortality curves are available as above, these doses and their ratio can be computed with a known accuracy. The "standard safety margin" has the added advantage of allowing for differences in the slope of the therapeutic and toxic curves and is not dependent upon the use of the same dosage unit (logarithmic or arithmetic) for both curves.

This procedure can be adapted readily to the evaluation of anthelmintics, using the mortality of the nemas in the rat as the measure of therapeutic effectiveness and mortality of the rat for the determination of toxicity. If the susceptibility of the rat to the drug is modified by the infection of worms, the toxicity should be determined with rats which have been inoculated in the same manner as those used in the therapeutic tests. The 99 and 1 per cent levels have the limitation that they are quite sensitive to errors in the slope of the dosage-mortality curve and hence are comparatively inefficient. They often represent extrapolations from the experimental data, and at its lower end the curve of toxicity may be modified by other factors as we have seen. It would be desirable, therefore, to alter the dosages to be compared from 1 and 99 per cent to 5 and 95 per cent respectively.

Sometimes drugs may be tested where the dose killing 5 per cent of the host is *smaller* than that killing 95 per cent of the parasite. In terms of the "safety margin" this would necessitate negative indices. The concept can be simplified, we believe, by using the ratio of the doses directly ($\times 100$) rather than the difference between the ratio and 1. We would propose defining the chemotherapeutic index as

$$C.I. = 100 \left(\frac{LD5}{ED95} \right) = \text{antilog } (2 + \log LD5 - \log ED95)$$

where LD5 is the lethal dose of drug killing 5 per cent of the host and ED95 is the "effective" dose killing 95 per cent of the parasites. A drug for veterinary use should have an index well over 100. From the equations for Figs. 2 and 3, the chemotherapeutic index for carbon tetrachloride has been determined as

$$C.I. = 100 \left(\frac{.2809}{.2036} \right) = 138 \text{ per cent.}$$

The proposed chemotherapeutic index is an estimate subject to the same errors as the two dosages from which it is computed. The resultant error of the index is complicated by unequal upper and lower limits in both numerator and denominator. In Fig. 3 the curve and its limits of error have been extended to intersect the probit corresponding to 5 per cent kill, indicated by a horizontal broken line. It is at once evident that in terms of the log-dose the lower limit is much further from the most probable value than the upper limit. This discrepancy is considerably less when the dosage-mortality curve is based upon more observations and when its mean mortality in probits approaches the value at which the dose is to be estimated, as in that for nematode mortality. Thus the LD5 for the toxic dose to the host of 0.281 cc

was determined within limits of 0.166 and 0.350 cc at odds of about 2 in 3, while the ED95 for the toxic dose to the parasite of 0.2036 cc fell between limits of 0.1964 and 0.2119 cc for the same odds.

Foster (5) has given a method for calculating the error of the standard safety margin from the upper and lower limits for both dosages. A simpler alternative based upon Eq. (28) in ref. (2) may be suggested for use where the dosage-mortality curves are computed in terms of probits and log-dose. Average variances for the log-LD5 and for log-ED95 are determined from the equation

$$V(X) = \frac{V(b)(Y - a)^2 + V(a)[b^2 - V(b)]}{[b^2 - V(b)]^2},$$

where $V(a)$ and $V(b)$ are the variances for position $a (= \bar{y})$ and for slope b respectively. The values computed from the dosage-mortality curve for the host are substituted in the above equation with $Y = 3.3551$ to obtain $V(X)_1$ and the equation is then solved again with the values from the dosage-mortality curve for the parasite and $Y = 6.6449$ to obtain $V(X)_2$. The upper and lower limits of the chemotherapeutic index (C.I.) for 1 standard error may then be calculated as

$$\text{C.I.} \pm \text{antilog} \sqrt{V(X)_1 + V(X)_2}.$$

Substituting the values from the present experiment, we find the limits were

$$138 \pm \text{antilog} \sqrt{0.026212 + 0.000272} = 138 \pm 1.454 = 200 \text{ and } 95 \text{ per cent.}$$

Although the toxicity of the drug to the parasite was determined within reasonably close limits, the relatively large error in the LD5 for the host limited the precision of the chemotherapeutic index. It is evident that for the best results, the dosage-mortality curve for the host should be comparable in accuracy to that for the parasite.

The dosage-mortality curves may be used for another, simpler comparison if so desired. Having determined the dose which will produce the desired kill of parasites, one may compute the expected mortality of the host at this dose. Thus, if 99 per cent kill of nematodes represents the therapeutic effect that is wanted, a dose of 0.254 cc of carbon tetrachloride is required. Substituting the logarithm of 0.254 or -0.595 in the equation for rat mortality, the expected mortality in probits is $y = 5.040 + 5.76(-0.595 + 0.259) = 3.105 (\pm 0.871)$. This represents an expected mortality of 2.9 per cent of the rats, falling within limits of 0.3 and 15.3 per cent.

EFFECT OF NUMBER OF INFECTIVE LARVAE INJECTED

The relation between the number of parasites and the efficacy of the drug has been tested in a preliminary experiment. Ten rats were each injected with the standard number of 500 infective larvae, 4 with an average of 1060 larvae and 3 with approximately 2120 larvae per rat. Eight days later 5 animals in the first group and all of those in the last two groups were treated with 0.26 cc of carbon tetrachloride in mineral oil (q.s. 0.5 cc) which would be expected to kill over 99 per cent of the parasites. Although more mature nematodes were recovered without treatment than in the controls for the first experiment, no parasites survived in the rats receiving the standard number of larvae and only 4 parasites in one of the 4 rats receiving twice the standard number (Table 5). With a fourfold increase in infection only one parasite was recovered from one rat out of three. While the action of the drug at this dosage was relatively independent of the number of infective larvae, doses below the therapeutic level may well be more sensitive to the size of

TABLE 5.—Relation of size of inoculation of *N. muris* to effectiveness of a dose of 0.26 cc of carbon tetrachloride diluted to 0.5 cc with mineral oil. Weights of groups when treated averaged from 113 to 155 grams

	"Dose" of infective larvae			
	509 ± 82		1060 ± 171	2120 ± 341
	Controls	Treated	Treated	Treated
No. of worms postmortem	460	0	0	0
	503	0	0	1
	411	0	4	0
	442	0	0	
	454	0		
Mean	454	0	1	0.33

the infection. Hence, wide fluctuations in the number of larvae should be avoided in determining the toxicity of drugs in vivo to *Nippostrongylus muris*.

SUMMARY

Dosage-mortality curves have been determined experimentally for the toxicity of carbon tetrachloride to artificial inoculations of *Nippostrongylus muris* in albino rats. When percentage kills were transformed to probits, the observations could be plotted linearly against the dose or log-dose, a relation similar to that observed for anthelmintics on parasitic nemas in vitro and for lethal drugs on other multicellular organisms. Since the percentage mortality was estimated from parallel untreated control groups, the standard calculation required certain modifications which are described.

The toxicity of the drug to albino rats was determined with a similar dosage-mortality curve, so that the dose of carbon tetrachloride killing predetermined percentages of both parasite and host could be computed with a measurable precision. Based on these curves, two methods for evaluating anthelmintics are proposed, (1) a redefined exact chemotherapeutic index computed from the ED95 and the LD5 with a known error and (2) the expected host mortality corresponding to the required therapeutic effect.

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CAN LARVAE OF *COCHLIOMYIA AMERICANA* C. AND P. MATURE IN CARCASSES?

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Cochliomyia americana C. and P. is normally an obligatory parasite causing myiasis in mammals. In nature an infested animal if left untreated becomes subject to multiple reinfestations, which usually cause the death of the animal. Animals with simple infestations may die from other causes. It is important to know whether the immature larvae remaining in these dead animals can develop to maturity.

Experiments were conducted at Valdosta, Ga., in 1936-39 to determine what age a *Cochliomyia americana* larva must reach in a live animal in order to complete its development in a carcass.

EXPERIMENTAL METHODS

The technique used was simple. Animals were infested with newly hatched larvae and then sacrificed after various periods. In each test one or more animals were placed outdoors where they were exposed to carrion-feeding insects and usually at the same time at least one animal was placed in a screened room protected from attack. Each carcass was placed on a board on sand in a rectangular galvanized metal pan 6 inches deep. The outdoor pans were kept under sheds to protect them from rain. The sand in each pan was sifted daily, and the larvae were spread on a flat sheet of metal. All larvae of *Cochliomyia americana* were removed and placed in sand in jars. Emerging flies were placed in fly-rearing cages and tested for egg laying. Fifteen tests with larvae of 5 ages were conducted in a screened room and 24 tests with larvae of 8 ages were conducted outdoors.

RESULTS

The data accumulated are summarized in Table 1.

Under protected conditions *Cochliomyia americana* larvae develop readily in carcasses even when only 24 hours old at the death of the host animal. Younger larvae may continue their development, although in the few tests conducted no larvae 15 hours or 20 hours old matured. However, Melvin and Bushland* have been able to rear such larvae to maturity on an artificial medium composed of ground meat and blood. In fact, the present procedure in rearing stock in laboratories conducting research on screwworm problems is to feed the larvae on such medium.

Much more important is the fate of the larvae when the carcass is exposed to other carrion feeders such as occurs under natural conditions when animals die as the result of screwworm attack. Under such conditions larvae 24 hours old at the death of the host may be recovered, but they are not sufficiently mature to develop to the adult stage. Larvae 30 hours old were found in one test to mature, pupate, and emerge as flies, but in that test the blowfly population was exceptionally low. Larvae 48 hours old have a greater chance of maturing, whereas a high percentage

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* Melvin, Roy and R. C. Bushland 1936 A method of rearing *Cochliomyia americana* C. and P. on artificial media. U. S. Dept. Agric., Bur. Entom. and Plant Quar. ET-88. 2 pp. Mimeographed.

TABLE 1.—*Development of Cochliomyia americana* larvae of various ages in carcasses screened and unscreened from attack by carrion-feeding insects

Age of larvae at death of host: Hours	Number of tests	Average number of larvae placed in carcass (approximate)	Average number of larvae recovered	Degree of emergence and egg laying of recovered larvae
Carcass screened				
15	3	300	0
20	2	500	0
24	5	340	95	Flies emerged and laid viable eggs in two out of four tests.
30	1	150	25	Flies deposited viable eggs.
48	4	237	181	High fly emergence and viable eggs always laid.
Carcass unscreened				
24	6	400	9	No flies emerged.
24	6	400	0
30	2	175	5.5	Flies emerged in one test and deposited eggs.
37	2	150	1	One fly emerged in one test but no eggs were deposited.
48	5	420	25.8	Flies emerged in three out of five tests and deposited viable eggs in two out of three tests.
54	1	500	329	Considerable emergence and many viable eggs deposited.
60	3	250	196	Considerable emergence; flies not tested for eggs.
76	4	Larvae recovered		Flies emerged, slightly subnormal in size but deposited viable eggs.

of those 54 hours old or older are fairly certain to mature and develop into flies that can deposit viable eggs.

There was no indication that the number of larvae per wound had any effect on the number of mature larvae recovered. It was found that larvae would continue to develop in carcasses even outdoors, but unless they were mature by the third day after death of the host they had little chance of continuing development, owing to competition with the many carrion-feeding larvae. Larvae 24 hours old and in the early second stage were observed to molt to the third stage by the third day after death of the host, but even if recovered these young third-stage larvae never developed to adults. Under outdoor conditions carrion-feeding larvae usually overran the carcass by the third day after death of the animal and *Cochliomyia americana* larvae could no longer be observed even though they had been seen as vigorously active 2 days after death of the host.

THE RELATIONSHIP BETWEEN THE INTESTINAL SIZE OF YOUNG
MICE AND THEIR SUSCEPTIBILITY TO INFECTION WITH
THE CESTODE, *HYMENOLEPIS NANA* VAR. *FRATERNA*

JOHN E. LARSH, JR.¹

Several workers have noticed that very young mice are more resistant to infection with the cestode, *H. nana* var. *fraterna*, than are mice between two and three months of age. Woodland (1924) observed that the smallest and the largest mice are least liable to be infected naturally. Later, in controlled experiments, Shorb (1933) and Hunninen (1935) showed that considerably fewer worms develop in mice about one month old, than in those of the most susceptible age (about two and one-half months old). Although the differences are not as striking, the writer has recently noted this same relationship in a series of experiments involving young mice, most of which were 21 to 25 days old. The percentage development of cysticercoids in these mice was about six compared with about eight to ten in mice two and one-half months old. Of the suggestions offered to explain this difference, Hunninen's seems most plausible. In his opinion, the shortness of the intestine and the small size of the villi in the very young mice act as a mechanical disadvantage to the normal hatching and penetrating activities of the onchospheres. If this is really the reason for their greater resistance to infection with this tapeworm, any factor that would increase their intestinal size should make them more susceptible.

It has been firmly established that the anterior pituitary of higher animals has a special relation to bodily growth and splanchnomegaly. The only figures that were found which showed a specific action of this gland on the development of intestinal tissue are from experiments with juvenile pigeons (Schooley et al., 1937) in which the response was accredited to the hormone, prolactin. By repeated injections of this hormone after hypophysectomy, the body weight of the pigeons was increased by eight per cent, intestinal length by 15 per cent, "empty weight" (weight after contents are removed) of intestine by 10 per cent, and villus length by 17 per cent over the corresponding values found in unoperated controls. It seemed desirable to test the effect of this hormone on the development of intestinal tissue in young mice to determine whether a similar increase could be produced, and, if produced, whether it would increase their susceptibility to the tapeworm.

DESCRIPTION OF EXPERIMENTS

At various intervals, four separate experiments of the same kind were carried out. In each, there were three groups of mice. Two of the groups were about 25-26 days old at the beginning of the experiment and the other was about 65 days old. All the mice were checked at least three different times during the experiment by the Lane technique to make certain they were not previously infected. Beginning with the 26th day after birth, one group of the younger mice received a series of ten

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daily 0.1 cc injections of prolactin² which were administered subcutaneously over the pectoral muscles (Schooley et al., 1941). The other group of younger mice served as untreated controls. The third group of older mice was included as a control on the degree of resistance to infection of the younger mice. On the day following the last prolactin injection (when the younger groups were 37, the older one, 76 days old), all the mice were given by stomach tube a test dose of eggs of *H. nana* var. *fraterna*, which had been isolated from fecal pellets stored in tap water at room temperature for 48 to 72 hours. All the mice were sacrificed 93 hours after receiving this test infection, and the number of cysticercoids that had developed was used as an index of infection (Hunninen, 1935). Determinations were made on both experimental and control mice to find whether the prolactin had affected the intestinal size. With the intestine under approximately uniform tension, its length, from pylorus to cecum, was measured to the nearest centimeter on a meter stick. The "empty weight" was recorded after the mucus was removed—a procedure preparatory to cysticercoid enumeration. The length of the villi was measured from prepared microscopical sections of the duodenum 2–3 cm below the pylorus; measurements were taken in 10 different radial planes over at least 50 sections, and the average used.

As shown in the table, all four groups of the 41-day-old control mice had a considerably smaller percentage development of cysticercoids than did the older, 80-day-old controls, which agrees in general with the results of similar comparisons mentioned above.³ On the other hand, three of the four groups of the younger mice that received prolactin treatment had percentages much higher than those of the untreated mice of the same age. In fact, these percentages were about the same as those of the second control group of 80-day-old mice. The other group that received prolactin (A of experiment 2), however, had a much smaller percentage development, which, in fact, was very much less than in any of the controls of the same age.

The response to prolactin treatment in all four experiments was striking. In the young mice treated in this way, there was an approximate 15 per cent gain in length of the intestine, a 54 per cent increase in "empty weight" of the intestine, and a 21 per cent increase in length of the intestinal villi over the corresponding values found in the untreated mice of the same age. In fact, the intestinal size (except for villus length) of the treated mice was almost the same as that of the mice in the older control group which were nearly twice their age.

DISCUSSION

The data given above indicate that prolactin affects very young mice in much the same way as young pigeons by increasing the length of the intestine, its "empty weight," and the size of the villi. This results in a much greater intestinal surface. Associated with this increased size of the intestine was an increased percentage development of the cestode, *H. nana* var. *fraterna*, in three of the four groups. In the other prolactin group (A of experiment 2) the resistance to infection was so much

² 100 prolactin units per cc prepared and standardized by the Armour Laboratories in accordance with the technique of Riddle et al (1933).

³ It is interesting to note that the highest cysticercoid percentages (around 5) in mice two and one-half months old are only about half those recorded in the earlier study, although in both cases stored eggs were used for infections. The only explanation that can be offered for these differences is that the worms used in the two series of experiments were from entirely different sources.

TABLE 1.—Showing the relationship between the increased intestinal size of young mice given prolactin and their susceptibility to infection with the cestode, *H. nana* var. *fraterna*

Experiment No.	No. of mice	Range in length of intestine from pylorus to cecum (cm)	Range in "empty weight" of intestine from pylorus to cecum (gm)	Average length of villi (microns)	Egg dose	Total No. of cysticercoids	Range in No. of cysticercoids	Percentage development
A. Young mice (41 days old) treated with ten injections of prolactin								
1	8	38.5-45.0	0.55-0.94	430	1850	788	84-138	5.0
2*	8	43.2-50.1	0.72-0.95	438	1050	60	0-21	0.7
3	7	41.0-49.0	0.60-0.82	434	1670	589	78-92	5.0
4	7	43.0-49.0	0.60-0.85	427	1350	404	52-80	4.9
B. Young mice (41 days old) not treated with prolactin								
1	8	34.0-41.0	0.40-0.68	350	1850	416	39-63	2.8
2	8	38.7-44.2	0.31-0.69	360	1050	304	20-56	3.6
3	7	33.0-40.0	0.34-0.65	335	1670	356	15-70	3.0
4	7	33.0-40.0	0.36-0.49	365	1350	212	23-55	2.2
C. "Optimum" age mice (80 days old)—the most susceptible group								
1	8	35.0-47.1	0.67-0.78	495	1850	786	81-134	5.3
2	8	39.0-52.0	0.69-0.87	521	1050	428	48-82	5.0
3	7	43.0-51.0	0.68-0.84	502	1670	631	87-148	5.3
4	7	43.0-47.0	0.68-0.75	501	1350	529	69-102	5.5

* See discussion in text.

greater than in the controls of the same age that it seems probable it was due to an immunity transferred from the mother (Larsh, 1942). In spite of the fact that all mothers of mice used in these experiments were examined by the Lane method, there is no way of ruling out a previous infection lost before the experiment began. It is possible that there are other factors in this resistance which are affected by the prolactin, but the most reasonable explanation for the higher percentage development of cysticercoids in the other three prolactin groups is the increased length and much greater surface of their intestines over that in the untreated mice of this age. As Hunninen has pointed out, the ingested eggs of this tapeworm pass through the intestine at a rate which allows only a certain number to hatch, so that in very young mice with a shorter intestine, fewer cysticercoids might be able to hatch and penetrate the intestinal wall than in older animals. In addition, he thought that the smaller villus size in these mice might act as an obstacle to normal cysticercoid development. This was suggested because in older mice the cysticercoids are found in the body of the villi, while in the very young mice they are deep in the mucosa or at the base of the villi. The fact that in very young mice prolactin both increases the size of the intestine and reduces the resistance to infection with *H. nana* var. *fraterna* seems to support Hunninen's theory.

SUMMARY

It has been generally recognized that very young mice are more resistant to infection with the cestode, *Hymenolepis nana* var. *fraterna*, than are mice between two and three months of age. The suggestion has been made that this resistance may be the result of the shortness of the intestine and the small size of the villi which serve as a mechanical obstruction to the normal hatching and penetrating activities of the onchospheres. To test this hypothesis, young mice were given repeated injections of the hormone, prolactin, which has been shown to have a specific action on the development of intestinal tissue in young pigeons. Young mice that received these injections showed a striking increase in the size of the intestine over that found in controls of the same age. Associated with this was an increased percentage development of cysticercoids. It is suggested that this increased susceptibility of the very young mice that were given prolactin was due to the increase in intestinal size.

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STUDIES ON *PROTECHINOSTOMA MUCRONISERTULATUM*, N. G.,
N. N. (*PSILOSTOMUM REFLEXAE* FELDMAN, 1941), A
TREMATODE (ECHINOSTOMIDAE) FROM
THE SORA RAIL*

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In October, 1939, 12 of 39 *Stagnicola palustris elodes* (Say) which were collected in a marshy area near Black Creek, Wisconsin, were infected with a species of large echinostome cercaria. It was found that the metacercaria would develop in various species of pulmonate snails, and with further collections in the fall of 1940 and in 1941, the adult stage was cultured in young albino mice. In October, 1941, 6 adult worms were taken from the lower ileum of a field host, *Porzana carolina* (L.). With a description of those examples from the field host, the study was concluded and a manuscript was nearing completion when a paper by Feldman (1941) was published on the morphology and life cycle of the same species. Comparison of the two studies revealed minor points on which observations differed, and some additional data which Feldman's paper did not contain. The purpose of the present report is to record these supplementary data.

The cercaria which Feldman obtained from *Stagnicola reflexa* was declared to be identical with *Cercaria reflexae* Cort, 1914, and the adult which was obtained from experimentally infected chicks was thought to be a member of the genus *Psilostomum* Looss, 1898. Thus this species was introduced into the literature as *Psilostomum reflexae*. For several reasons to be discussed later, mainly because of its crown of spines, this species cannot be a member of the genus *Psilostomum*. It represents instead a new genus. Also, there is no justification for declaring the cercaria identical with *C. reflexae* Cort. It resembles *C. reflexae* but differs from it markedly, especially in size and spination. For *Psilostomum reflexae*, the new name *Protechinostoma mucronisertulatum*, n. g., n. n. is proposed.

THE LARVAL STAGES

The cercariae encyst in *Stagnicola palustris* and other species of pulmonate snails. *Lymnaea stagnalis*, *Physa* spp., *Helisoma trivolvis*, and *Fossaria* sp. as well as *S. palustris* all were infected in the laboratory, and individuals of each of these species were found to carry up to 40 metacercariae when collected from natural pools where cercariae were known to be present. The cercariae enter the snails through the respiratory orifice and encyst in the adjacent portions of the mantle, in the walls of the respiratory sac, or in masses that lie more or less free in the respiratory cavity. Attempts to infect fish and tadpoles were unsuccessful.

The descriptive data which follow on the larval stages include only those observations which either add to, or clarify the descriptions by Feldman.

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The Redia

(Figs. 3-4)

Mature stages very large, many over 2 mm in length. Each large example contains about 10 cercariae in various stages of development. Body almost unpigmented; the oldest ones sometimes bearing scattered minute dark orange colored granules. Collar regular and generally conspicuous in living specimens; not divided into distinct lobes. Birthpore close behind collar and often crowded into it. Pharynx relatively small, not capable of withdrawal behind collar. Flame cells in 2 groups on each side; one group at base of appendages, the other half-way between appendages and collar. Pulsating bladder on each side just anterior to appendages. Number of flame cells variable with size of redia; 9 in each group were observed in a specimen about 0.5 mm long, and in a well-extended specimen, 1.6 mm in length, there were 15 in each group. Immature cercariae were not observed free in the tissues of the snail. In larger hosts several thousands of rediae often were present.

The Cercaria

(Figs. 1-2)

Noteworthy features of the living cercaria are its relatively large size, slightly yellowish opaque cystogenous glands which fill most of the body, relatively small suckers, narrow excretory siphons containing numerous small granules, and large fins on both dorsal and ventral sides of the long and powerful tail. Fleshy collar inconspicuous but under favorable conditions, its crown of spines is easily observed. Pattern of collar spines identical with that of adult. Cuticular spines minute and evident only in preacetabular region. Cephalic ducts dorsal to oral sucker about 24 in number. Excretory bladder two-chambered; main excretory canals extend forward as enlarged siphons to anterior end and then extend unbranched back to extreme posterior end. Flame cells appear to be in groups of 3, with 12 anterior to bifurcation of esophagus, 6 in acetabular region, and 24 posterior to acetabulum on each side. Bifurcation of caudal excretory canal at junction of first and second fourths of tail. Dorsal fin low at base of tail, absent or extremely low along a short middle portion, and high along distal third. Ventral fin continuous from base to tip of tail but low near middle. Dimensions of average extended fixed specimens: body length, 0.50 mm; width at acetabulum, 0.16 mm; oral sucker, 56 by 50 μ ; diameter of expanded acetabulum, 80 μ ; contracted, 60 μ ; pharynx, 30 by 24 μ ; tail length, 0.67 mm; width of tail at base, 64 μ ; greatest height of tail fin, 22 μ .

The Metacercaria

Cysts fairly uniform in size and shape. Even when crowded they are spherical, 0.252 to 0.280 mm in diameter. Cyst wall smooth and transparent, 17 to 20 μ in thickness. Body of metacercaria flexed ventrally and coiled. Growth rapid first week, slower but continuous for next 2 to 3 weeks. At 3 to 4 weeks, cuticular spination is well developed on all except post-acetabular dorsal region of body; collar spines and anterior body spines of equal size, 4 μ long; gonads well developed, testes almost as large as oral sucker; oral sucker, 60 by 50 μ ; diameter of acetabulum, 90 μ ; pharynx, 40 by 34 μ ; length of worm, about 0.6 mm; width at acetabulum, 0.22 mm; excretory system much advanced with lateral branching canals along siphons from excretory bladder to level of pharynx; all main excretory canals and bladder greatly dilated. No appreciable changes occur in the metacercariae after 4 weeks and they remain viable in the snails for at least 5 months.

THE ADULT

In agreement with Feldman's experience, it was found that the range of conditions under which *P. mucronisertulatum* will develop in laboratory hosts is very narrow. Feldman found that of several species of birds and mammals used, mature worms could be cultured in young chicks only, and even in these hosts the results were always uncertain. He also reported that metacercariae are infective after only 5 to 7 days of encystment, and that the adult worm is mature 5 days after infection. In view of the additional growth and maturation of metacercariae for at least 2 weeks after the first 5-day period, it seems doubtful if many are infective in so short a period.

The experiments summarized here were completed before adult worms were discovered in a field host. The field host (sora rail) being a bird, and Feldman's successful experiments being limited to young chicks, it is noteworthy that numerous

attempts to obtain infections in pigeons and chicks were unsuccessful while in 5 days after infection, albino mice produced mature worms about the same size as those from the bird hosts. Not all attempts to infect mice were successful, however, and feeding experiments with other mammals (5 albino rats, 5 hairless rats, 2 rabbits, 2 bats, and a guinea pig) were negative. Within a week after weaning, 10 mice were fed 100 to 200 metacercariae that ranged from 3 to 4 weeks of age. Five of the mice were found to contain no worms 2 days after feeding, and 1 was negative on the 5th day. From 3 of the remaining mice, 1, 1, and 14 worms respectively were recovered on the 2nd day after feeding. The 10th mouse was fed about 100 metacercariae on each of 4 consecutive days and was examined on the 5th day. Concentrated in the most distal 10 mm segment of the ileum, there were 72 worms of various stages of maturity, ranging from the very small 24-hour stage to mature worms with 1 or 2 eggs in the uterus.

In pigeons and young chicks the metacercarial cyst wall was undigested and the encysted metacercariae were invariably dead when they were passed in the feces. Apparently cysts sometimes were fractured, however, enabling the worm to escape. One living worm was found in the middle small intestine of a pigeon on the 2nd day after it had been fed metacercariae. In the feces of this pigeon there were 200 to 300 encysted metacercariae all of which were dead when passed. In bats most of the metacercariae excysted but were expelled in less than 3 days, and most of them were dead in the stools when passed. Metacercariae likewise excysted in rats but apparently did not live more than 1 or 2 days. The rats were fed 300 to 400 cysts each and were examined after 1 to 8 days with negative results in all but one instance. A black-eyed hairless, an albino hairless, and 2 common albinos were examined 24 hours after receiving metacercariae and while empty open cysts were found in each of them, only the black-eyed hairless contained living worms. In it there were over 300 worms, all in the lower ileum.

The passage of metacercarial cysts undigested through the entire digestive tract of some species of experimental hosts, notably the pigeon, while in others all of the metacercariae generally are liberated, suggests the absence of specific enzymes for digesting the cyst wall as an important factor in the limitation of hosts both in the laboratory and in the field.

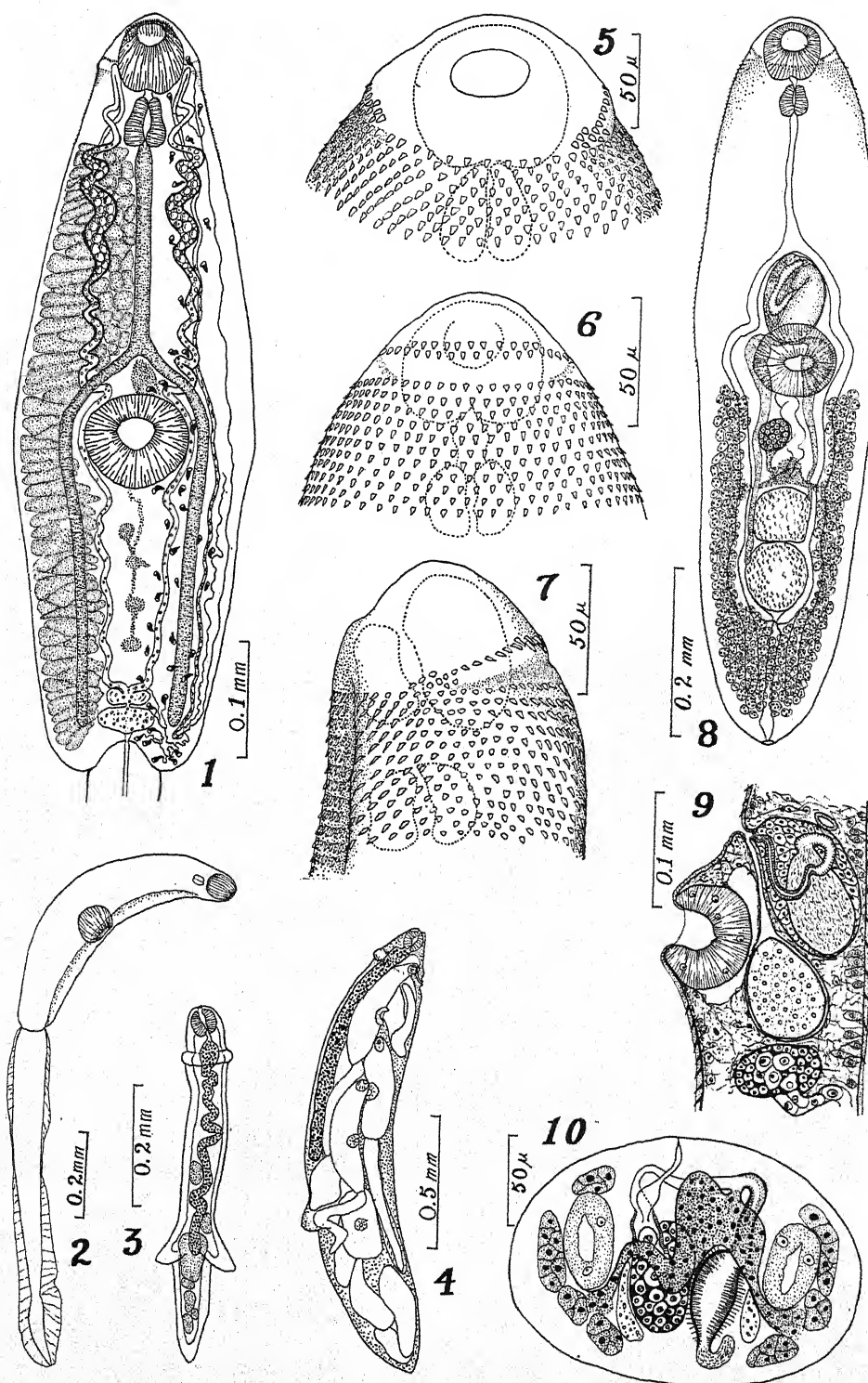
A pied-billed grebe (*Podilymbus podiceps*), 2 coots (*Fulica americana*), 6 muskrats (*Ondatra zibethica*), and 7 shrews (*Blarina brevicauda*), all known to inhabit and feed regularly in the immediate region where cercariae of *P. mucronisertulatum* were abundant, were uninfected with this species. The rail from which 6 specimens were taken was killed in the same watershed but about 10 miles distant from pools where cercariae were known to occur.

Studies on numerous specimens from experimentally infected young mice, 6 specimens from the rail, and 8 specimens which Feldman obtained from young chicks have brought to light several features of the adult stage that should be added to the earlier description.

Additional Data on Adult Morphology

(Figs. 5-10)

Cuticular spines extend almost to posterior end on ventral side but only to region of ovary on dorsal side of mature worms. Their arrangement is quincuncial and very regular. Fleshy crown weakly developed and not always evident on fixed specimens, especially when flattened. Crown spines barely if any larger than adjacent body spines. Typical number of crown spines is 51, with



very little variability in number and arrangement. Crown pattern: angle spines 7 in number, 3 anterior and 2 posterior ones in alternating rows that form a continuation of the lateral group, and 2 somewhat smaller ones in the angle between the body spines and the crown; lateral crown spines in a single row, 8 in number; dorsal group in 2 alternating rows, 11 in oral row, 10 in aboral; aboral row continuous with laterals. In young specimens just reaching maturity sperm ducts are dilated forming a vesicula seminalis externa on each side in region of acetabulum. Cirrus sac mostly anterior to acetabulum but may reach its posterior border. Seminal vesicle large and not divided into chambers; pars prostatica about half as long as cirrus sac; prostate gland cells occupy roughly half of cirrus sac. Cirrus unarmed. Laurer's canal to right of midline, short, without opening to outside. Seminal receptacle absent.

Protechinostoma n. g.

Diagnosis: Echinostomidae with small, linguiform, spinous body. Collar weakly developed, bearing a crown of spines barely if any larger than body spines. Crown spines arranged into angle, lateral, and dorsal groups as in *Echinostoma*, *Hypoderaeum*, and others. Angle and dorsal spines arranged in alternating pattern; the laterals in a single row. Acetabulum larger than oral sucker but relatively small for size of body. Cirrus sac anterior to the posterior border of acetabulum. Cirrus unarmed; prostate gland present; seminal vesicle single-chambered. Testes spherical to cuboid with smooth margins, situated about midway between acetabulum and posterior end. Ovary spherical with smooth margins. Uterus short, containing relatively few eggs. Vitellaria extend from posterior end to level of acetabulum. Seminal receptacle absent.

Type species: *P. mucronisertulatum*.

DISCUSSION

The new genus *Protechinostoma* stands in an intermediate position between the least specialized of the true echinostomes and the rather numerous echinostome-like genera (including some psilostomes) in which a distinct collar with a ventrally interrupted crown of spines is lacking. Of the true echinostomes it appears to stand nearest the genus *Hypoderaeum* Djetz, 1909 (1910). In both genera the crown spines are small, numerous, and somewhat similarly distributed on a weakly developed fleshy collar. In *Hypoderaeum*, however, the fleshy collar is more muscular, the acetabulum is large, the uterus is very long, and the testes are elongate as compared with *Protechinostoma*. In one species of *Hypoderaeum* (*H. mainpuria* Verma, 1935), the collar spines are so minute that they were overlooked entirely when the first description of it was made. The crown spines and the feeble muscular collar of *Protechinostoma* distinguish it from all echinostome-like genera in which either the spines or the crown is lacking. If in examining *Protechinostoma* spp. either or both of the crown structures were overlooked or misinterpreted, they might be confused with *Pseudechinostomum* Odhner, 1911, *Aequistoma* (Shchupakov,

Protechinostoma mucronisertulatum

Drawings made with the aid of a camera lucida.

- FIG. 1. Cercaria. Ventral view of body, unflattened.
- FIG. 2. Cercaria. Lateral view of extended fixed specimen.
- FIG. 3. Immature redia. Ventral view.
- FIG. 4. Mature redia. Lateral view, moderately contracted.
- FIG. 5. Anterior end of adult. Ventral view of an unflattened fixed specimen from a rail.
- FIG. 6. Anterior end of adult. Dorsal view of a small fixed specimen from a mouse. Glycerine mount under pressure.
- FIG. 7. Anterior end of adult. Lateral view of mature fixed specimen from a mouse.
- FIG. 8. Young adult. Ventral view showing characteristic dilated sperm ducts in a specimen from a mouse.
- FIG. 9. Adult. Composite sagittal section through the genital pore, cirrus sac, ovary, egg, metraterm, and acetabulum.
- FIG. 10. Adult. Composite transverse section through the ovary, Laurer's canal, vitelline reservoir, and oötype. Posterior view.

1936) Beaver, 1942, *Psilorchis* Thapar and Lal, 1935, or *Psilostomum* Looss, 1898. In *Pseudechinostomum* a well-defined crown is present but it is without spines, and differs otherwise from *Protechinostoma* by having a long uterus and a cirrus sac extending beyond the posterior border of the acetabulum. In the three other genera, crown structures are completely wanting. *Psilostomum* differs further from *Protechinostoma* in the shortness of its esophagus (Price, 1942). The crown structures of *Parorchis snipis* Lal, 1936, are like those of *Protechinostoma* but the two forms are otherwise dissimilar.

The resemblance between the larval stages of *P. mucronisertulatum* and *Cercaria reflexae* Cort, 1914, is at once apparent but does not reach the level of specific characters. *C. reflexae* is much the smaller and is without refractive granules in the excretory siphons. The most important difference between the two species, however, is the complete absence of crown spines in *C. reflexae*. Cort (1915) specifically points out that these structures are absent and there is no reason to suspect that they were overlooked. He noted that "The surface of the body to the region about half way from the acetabulum to the posterior end is covered with small spines arranged in rows and set closely together." If crown spines were present Cort would have seen them as easily as he saw the body spines. Sometimes allowances must be made for errors and omissions in different investigators' observations, but in this case the extreme allowances called for to bring *C. reflexae* into agreement with the cercaria of *P. mucronisertulatum* seem to be without justification.

SUMMARY

1. Additional data are reported on the cercaria, redia, metacercaria, and adult of *Psilostomum reflexae* Feldman, 1941.
2. It is found that this species is not a psilostome, but is instead an echinostome having a weakly developed crown. It represents a new genus for which the name *Protechinostoma* is proposed.
3. The cercaria which was originally reported as identical with *Cercaria reflexae* Cort, 1914, is shown to be a distinct species. A new name, *Protechinostoma mucronisertulatum*, is proposed for it.
4. A new laboratory host, the albino mouse, and a field host, the sora rail (*Porzana carolina*), are reported for the adult stage.

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THE LIFE HISTORY OF *LECITHASTER CONFUSUS* ODHNER
(TREMATODA: HEMIURIDAE)¹

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During the summer of 1941, we found a cystophorous cercaria developing in the marine snail, *Odostomia trifida*, collected from Eel Pond extension of Waquoit Bay, Massachusetts. Experimental determination of the life history of this cercaria has demonstrated that its adult stage is the hemiurid trematode, *Lecithaster confusus* Odhner, 1905, a widely distributed intestinal parasite of marine fishes. Copepods were found to serve as the second intermediate host.

A number of marine and freshwater cystophorous cercariae have been described, but very few of their life cycles are known. Those that have been traced experimentally are limited to species of *Halipegus*, a curious group of freshwater hemiurids which live in the oral cavity and eustachian tubes of amphibians. On the basis of morphological agreement between *Cercaria cystophora* Wagener and *Halipegus ovocaudatus*, Sinitsin (1911) states that Leuckart anticipated their genetic relationship but incorrectly assumed that frogs became infected directly, without the mediation of a second intermediate host. Sinitsin (1905) reported that metacercariae of *H. ovocaudatus* developed in nymphs of the dragon fly, *Calopteryx virgo*. Thomas (1934) described a cystophorous form, *Cercaria sphaerula*, which, when ingested by *Cyclops vulgaris*, developed as a metacercaria free in the body cavity of that copepod. Krull (1935) traced the life history of *H. occidua*, for which he described a cystophorous cercaria and showed that dragon fly nymphs served as second intermediate hosts. In determining the life cycle of *H. eccentricus*, Thomas (1939) observed that the cercaria, a cystophorous larva, was ingested by *Cyclops vulgaris* and *Mesocyclops obsoletus* and that the metacercaria developed in the body cavity of these hosts. He reported that when infected copepods were eaten by tadpoles, the young worms remained in the stomach until metamorphosis, after which they migrated to the oral cavity and eustachian tubes where they occur in adult frogs.

Although the life histories of no marine hemiurids have hitherto been traced experimentally, their metacercariae are known to occur in a variety of marine invertebrates, chiefly the small crustaceans. Dollfus (1923) recorded them from the copepods, *Acartia clausi* and *Centropages hamatus* and later (1927) identified as a species of *Dinurus*, the progenetic metacercaria which Bonnier had found in *Cerataspis monstrosa*. Lebour (1923) reported the metacercaria of *Hemiurus communis* in the body cavity of a species of *Acartia*. Hemiurid metacercaria have also been observed in such marine animals as chaetognaths and ctenophores, which probably are accidental hosts not ordinarily included in the life cycle. These and other references to hemiurid metacercariae are given by Thomas (1934).

Lecithaster confusus, whose life history is the subject of the present study, has

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been reported from a number of marine fishes. Linton (1940) lists as hosts of this trematode in the Woods Hole region: *Centropristis striatus*, *Clupea harengus*, *Etrumeus sadina*, *Pomolobus mediocris*, *Poronotus triacanthus*, *Scomber scombrus*, *Spheroides maculatus*, and *Tautoglabrus adspersus*. To this host list of *L. confusus* may be added the killifish, *Fundulus heteroclitus* and *F. majalis*, and the four-spined stickleback, *Apeltes quadracus*, which we have found to be infected in nature.

MATERIALS AND METHODS

Since an unlimited supply of the various stages in the life cycle of *L. confusus* was available, living material was studied as much as time permitted. Permanently stained material was found useful, however, for certain observations and measurements.

For experimental infections, we used sticklebacks from the Eel Pond (in Woods Hole and not to be confused with a part of Waquoit Bay which is about ten miles from Woods Hole), and copepods of the genus *Acartia* from Vineyard Sound since stages of *L. confusus* were not found in any of a number of copepods and sticklebacks collected from these localities. The copepods were placed in finger bowls of sea water containing infected snails. Half of the bowls were covered with cloth and placed under a stream of filtered sea water while the others were left uncovered, but with a continuous flow of water on the outside of the bowls to maintain a favorable temperature. Although the copepods lived longer in the uncovered dishes, they did not survive beyond nine days. During that time, specimens were removed daily to observe growth and development of the metacercaria. The excretory pattern of the adult was investigated in both young and mature specimens. To observe the full length of the long excretory tubules, it was necessary to flatten the worms and manipulate the cover glass so that a semi-lateral view was obtained.

OBSERVATIONS

Proof of the Life History of *Lecithaster confusus*

While we were unable to use laboratory-reared experimental animals, it was found that copepods and fish, collected from the localities mentioned above, were free from infection with stages in the life history of *L. confusus*. When animals from those localities were exposed to infection in the laboratory, they were subsequently found to harbor stages of *L. confusus* which agreed in every respect with those found in naturally infected hosts from Waquoit Bay. Furthermore, the incidence and intensity of experimental infection were significantly higher than in animals collected from Waquoit Bay where *L. confusus* probably is the most abundant trematode parasite of fish. Thus, of several hundred *Acartia* collected from Vineyard Sound and exposed to cercariae of *L. confusus*, practically all became infected, some with as many as 15 metacercariae, while no worms were found in any of 55 control copepods. Only ten per cent of the copepods collected at Waquoit Bay harbored natural infections of *L. confusus* and few of those contained more than one metacercaria each. Similar results were obtained in experiments with the definitive hosts; adults of *L. confusus* were recovered from all of 25 sticklebacks, collected from the Eel Pond at Woods Hole and fed infected copepods while 40 control fish from the same place were negative for that parasite. Experimental fish were fed naturally infected *Acartia* because of the difficulty of keeping experimentally infected copepods alive.

However, experimentally infected *Acartia* were examined daily for nine days and development of the metacercariae was followed carefully to a stage at which they were identical to larvae recovered from naturally infected copepods. It therefore seems highly improbable that we have confused stages in the life history of more than one hemiurid species.

In an extensive survey of fish parasites at Waquoit Bay, we have found only two hemiurids, *L. confusus* and *Hemiurus appendiculatus*. The metacercaria of the latter also occurs in *Acartia* but is so strikingly similar to the adult, an appendiculate form, that it could hardly be mistaken for the larva of *L. confusus*. A cystophorous cercaria, which resembles *Cercaria sinitzini* Rothschild, has been collected only once from Waquoit Bay and has not been available in numbers sufficient for an experimental study of the life history. It probably is the larva of *H. appendiculatus*, which is much less common in Waquoit Bay copepods and fishes than is *L. confusus*.

Description of Stages in the Life History

The redia

(Fig. 14)

Only rediae producing cercaria were observed. They are non-pigmented, elongate larvae with somewhat tapered ends. The largest ones observed were 2.5 mm long and 0.08 mm wide. Mature rediae are constricted at intervals and contain most of the mature cercariae in the expanded regions between constrictions. The posterior end of such a redia is frequently shrunken and degenerate (Fig. 14) with its supply of germ cells apparently exhausted. Since no birth pore was observed, mature cercariae probably escape by rupture of the redial wall in the regions distended with fully developed larvae.

The cercaria

(Figs. 1-11)

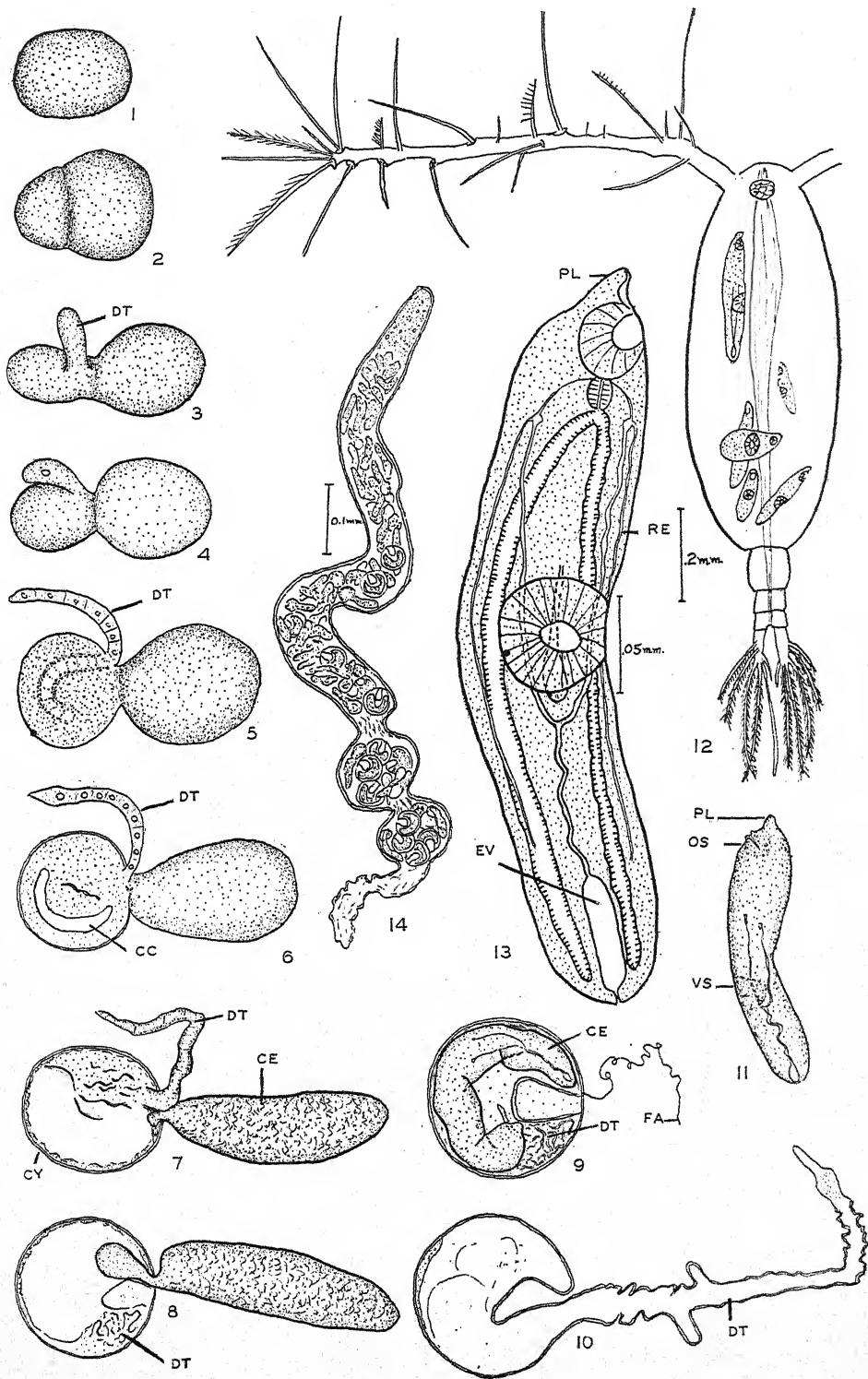
Specific diagnosis: Non-swimming cystophorous cercaria with discoid bulb or cyst 0.05 mm in diameter and having very delicate filament as only external appendage; delivery tube enclosed within cyst when cercaria emerges from snail; everted tube 0.15 mm long and 0.01 mm in diameter near cyst, distal half about 0.007 mm in diameter except slight enlargement near tip; everted tube with two short projections on opposite sides, one usually closer than the other to cyst. Cercarial body with smooth cuticle and short pre-oral lobe; average body length 0.085 mm, width 0.02 mm; structure poorly defined, even suckers being indistinct. Excretory vesicle a delicate Y-shaped tube with arms extending about half way to anterior end of body. Other structures indistinguishable in living cercariae.

Host: *Odostomia trifida* (Totten).

Locality: Eel Pond extension of Waquoit Bay, Massachusetts, U.S.A.

Fifty per cent of several hundred *O. trifida*, collected from Waquoit Bay, were found to be infected with the cercaria of *L. confusus*. The larvae escape in large numbers from snails isolated in the laboratory. Since they are extremely small and incapable of swimming, the cercariae are easily overlooked.

Observations on the embryology of the cercaria were made, following development from the germ ball to the stage which escapes from the snail. The germ ball is at first a subspherical mass of cells (Fig. 1) which soon becomes divided by a transverse constriction into two parts, the future bulb, cyst, or caudal vesicle, and the cercarial body. The delivery tube is first seen as a short outgrowth from the primordium of the bulb (Fig. 2). It gradually elongates (Figs. 3-6) and is seen



to consist of a column of cells. At this stage, a cavity appears in the cyst. With continued growth of the cyst, the delivery tube becomes hollow and is withdrawn into the bulb where it is seen as a closely coiled mass at one end of the cyst cavity (Fig. 8). Finally, the cercarial body withdraws into the cavity, the opening apparently becomes sealed, and the larva is fully formed. The delicate thread on the outside of the fully developed cercarial cyst probably is homologous with one of the various appendages described for other cystophorous cercariae; its origin was not determined in this study.

When the cercariae escape from the snail, they appear spherical but actually are slightly flattened. When mounted in sea water, light cover glass pressure causes excystment much as described by Willey (1930) for *Cercaria projecta*. In the process, the delivery tube is everted forcibly and the larva passes through it so rapidly that many observations were necessary to determine that the larva emerged at the tip of the tube rather than from one of the lateral projections. When the tube evaginates, the larva elongates as it shoots to the outside apparently as a result of intracystic pressure and elastic recoil of the tube. Passage of the larva through the delivery tube was observed by Willey (1930) for *Cercaria projecta*, Krull (1935) for *Halipegus occidualis*, and Rothschild (1938) for *Cercaria sinitzini*. It therefore seems likely that the process of excystment is much the same in all hemiurid cercariae although Thomas (1939) was unable to observe passage of the body through the delivery tube in the larva of *Halipegus eccentricus*.

While we have not observed excystment in the body of the copepod, the delivery tube probably pierces the intestine and the lateral extensions serve to anchor the tube in place until the larva passes through it into the body cavity of the host. This function of the delivery tube has been reported by Krull (1935) for the cercaria of *H. occidualis*. Due to the lack of penetration glands or even functional suckers in the cercaria, it is difficult to conceive any other means whereby it could reach the body cavity of the copepod.

The metacercaria

(Figs. 12 and 13)

Data concerning experimental infection of copepods have been presented above as evidence contributing to the proof of the life history. The metacercariae of *L. confusus* occur unencysted (Fig. 12) in the hemocoel of *Acartia tonsa* where they are quite active and move about freely. The cuticle is aspinose and lacks the segmented appearance and caudal appendage of the larva of *Hemiuris appendiculatus* which also occurs in the body cavity of *Acartia*.

Growth and development of the metacercariae in *Acartia* are rapid. In nine days, the larva (Fig. 13) increases in length from 0.08 to almost 0.4 mm, suckers

Lecithaster confusus

Figs. 1-9. Stages in development of cercaria from germ ball.

FIG. 10. Cyst showing everted delivery tube through which cercaria has emerged.

FIG. 11. Body of cercaria after escaping from cyst.

FIG. 12. Experimentally infected copepod containing six metacercaria.

FIG. 13. Nine-day metacercaria from *Acartia tonsa*.

FIG. 14. Redia.

CC—cyst cavity

CE—body of cercaria

CY—cyst

DT—delivery tube

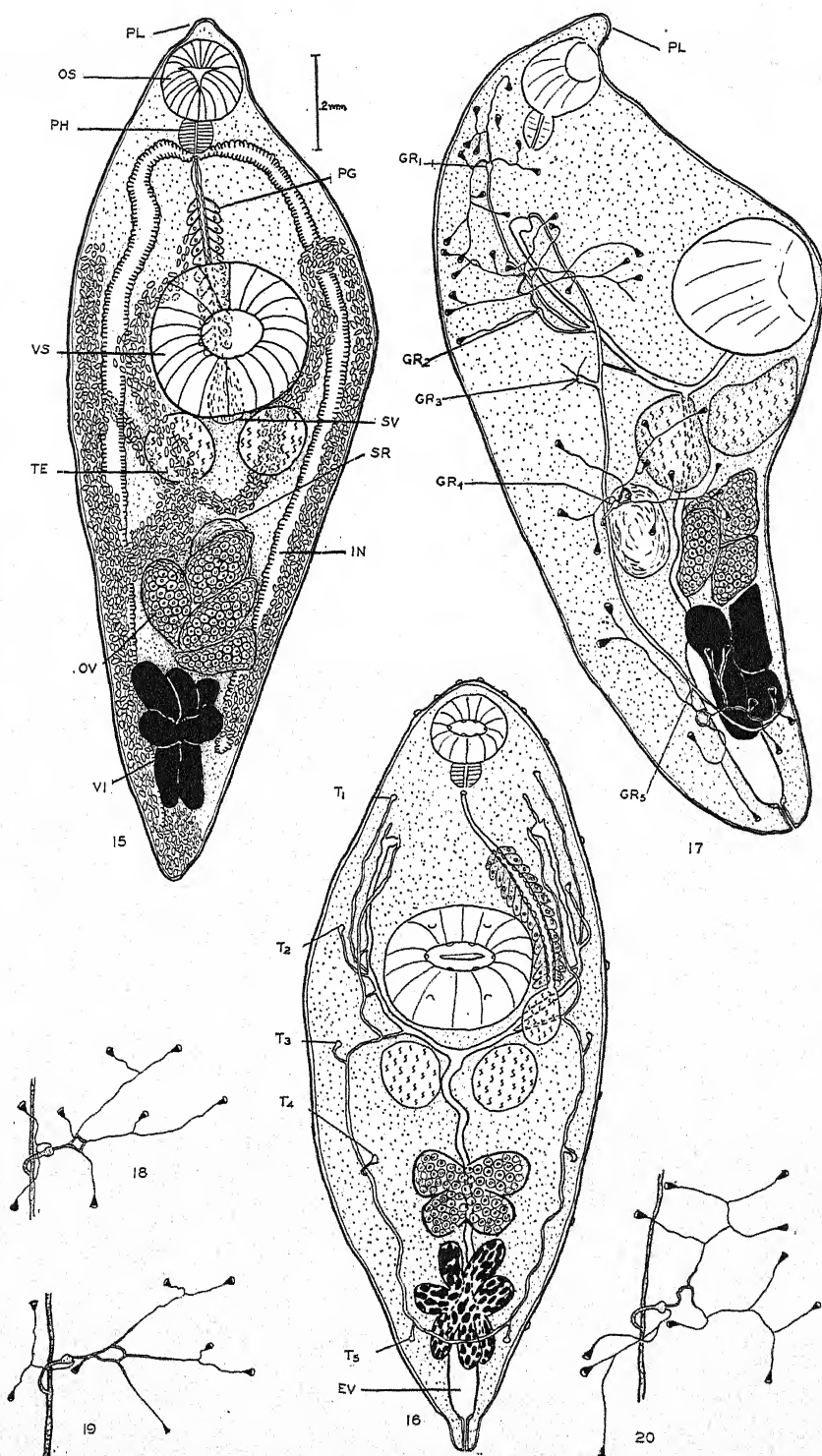
EV—excretory vesicle

FA—filamentous appendage

PL—preoral lobe

RE—recurrent excretory tube

VS—ventral sucker



become well formed, and the excretory and reproductive systems attain a fairly advanced stage of development. Average measurements in millimeters of moderately extended, living, 9-day metacercariae are as follows: body length 0.39, width 0.094; width of oral sucker 0.06, of ventral sucker 0.075; pre-oral lobe 0.02 long; pharynx diameter 0.028. A prepharynx is lacking and the intestinal ceca extend to the posterior end of the body.

In 9-day metacercariae, the Y-shaped excretory vesicle consists of an expanded posterior sac with a slender median tube extending almost to the ventral sucker and dividing to form two arms which terminate anteriorly at the sides of the pharynx. A delicate transverse tubule, uniting the slightly expanded terminations of the arms, represents the anterior commissure characteristic of many adult hemiurids. In *L. confusus*, however, this connection disappears and cannot be detected in the adult worm. The presence of an anterior commissure in the metacercaria of this species supports Yamaguti's (1934) opinion that certain genera lacking the commissure, viz., *Lecithaster* and *Hysterolecithoides*, form an aberrant group of the family HEMIURIDAE.

The adult (Figs. 15–20)

It is unnecessary to redescribe completely the adult of *L. confusus* since fairly complete accounts have been given by Odhner (1905), Looss (1908), and Linton (1940). These authors did not mention the small, contractile papillae occurring in the suckers and on the body; they are visible only in living material and even then may be contracted and consequently overlooked. Those on the body (Fig. 16) are dorsolateral in position and are seen clearly only in obliquely dorsal and ventral views of the worm. General morphological features of the adult *L. confusus* are shown in Figs. 15–17 which are self-explanatory.

DISCUSSION

Descriptions of the excretory systems of hemiurids are practically non-existent in the literature. Our observations on *L. confusus*, while incomplete, show that the system is extremely complex and apparently subject to considerable variability in the adult stage. Observations of the excretory pattern in both immature and mature adults indicate that some of the variations may be correlated with the size of the worm.

The excretory vesicle of the adult is much as described already for the metacercaria. A short canal leads from the vesicle to the pore which is situated at the posterior tip of the body. The expanded portion of the vesicle reaches almost to the anterior margin of the vitelline lobes. From that level, the vesicle continues as

Lecithaster confusus

FIG. 15. Mature adult.

FIG. 16. Adult showing principle excretory tubules.

FIG. 17. Lateral view of adult showing excretory system of one side.

FIGS. 18–20. Variations in Flame Cell Group 4.

EV—excretory vesicle
GR—numbered flame cell
group
IN—intestine
OS—oral sucker
OV—ovary

PG—prostate gland
PH—pharynx
PL—preoral lobe
SR—seminal receptacle
SV—seminal vesicle

T_n—excretory tubules serving
flame cell groups with
corresponding numbers
TE—testis
VI—vitellaria
VS—ventral sucker

a tube to the posterior margin of the acetabulum, there dividing into two arms, each of which extends anteriorly, lateral to the ventral sucker, and terminates at the pharyngeal level. Remnants of the anterior commissure are observed as blind tubes extending a short distance from the expanded anterior terminations of the vesicle arms. From these terminations, a pair of long recurrent tubules extend posteriorly as far as the vitellaria where they are united by a transverse commissure which crosses the body ventral to the vitelline mass. On each side of the body, there are five short secondary tubules, each terminating with a characteristic enlargement which is joined by smaller tubules from a group of flame cells. In Figs. 16 and 17, the secondary tubules are numbered from anterior to posterior ends of the worm and their associated flame cell groups are assigned corresponding numbers to facilitate description of the excretory system.

At the level of the ventral sucker, Tubules 1 and 2 join what we have interpreted as the arms of the excretory vesicle. Tubule 1 is long and serves the anterior-most group of flame cells on its respective side of the body; the short Tubule 2 is joined by the second group of flame cells which is located at the level of the ventral sucker. Tubules 3 to 5 join the recurrent excretory tubule and serve the posterior region of the body, their corresponding flame cell groups being approximately at the levels of the testes, ovary, and vitellaria respectively.

We were unable to determine the number of flame cells in Group 3 or the exact connections of certain flame cells and capillaries in Groups 1 and 2. There is considerable variation, not only in the number of flame cells and the patterns of capillaries and small tubules in the various groups, but also in the formation of secondary connections between the recurrent tubules and the arms of the vesicle. Variations in Group 4 were studied in considerable detail since other groups were more difficult to observe in their entirety. Figs. 17 to 20 show four modifications of the pattern observed in Group 4. It is seen that the tubule connecting most of the flame cells to the expanded end of the secondary tubule may or may not form a complete loop and the number of flame cells in the group varies from eight to eleven. Since Fig. 20 represents the condition observed in a large, mature worm and has the maximum number of flame cells, it probably represents the ultimate nature of the group although the loop observed in many specimens was lacking. It is possible that such loops, like the anterior commissure, are transitory in *L. confusus*. Fig. 19 suggests that they form by fusion of one of the flame cell capillaries with the tertiary collecting tubule.

The presence of one or more tubules connecting the arms of the vesicle and the recurrent tubule as well as the direct junction of two pairs of secondary tubules with the arms make it difficult to interpret the parts of the excretory system. For this reason, our description of the vesicle as being Y-shaped with arms reaching the pharyngeal level is regarded as tentative; we are by no means certain as to where the vesicle proper ends and the collecting tubules begin. In some respects the excretory system of *L. confusus* is suggestive of the "reserve" network of certain strigeids.

SUMMARY

The life history of *Lecithaster confusus* (Odhner) has been traced experimentally. The cercaria is a small cystophorous larva developing in elongate, constricted rediae in the digestive gland of the marine snail, *Odostomia trifida*, collected from Waquoit Bay, Massachusetts, U. S. A.

Cercariae are ingested by copepods of the genus *Acartia* and develop into unencysted, active metacercariae in the hemocoel of the copepod. Adult worms were recovered from the intestine of sticklebacks, *Apeltes quadracus*, which had been fed infected copepods. The definitive host list of *L. confusus* is extended to include *A. quadracus*; also the killifish, *Fundulus heteroclitus* and *F. majalis*, all of which were found to harbor natural infections.

Development of the cercaria from the germ ball has been traced and the excretory system of the adult is described. An anterior excretory commissure is present in the metacercaria but disappears in the adult stage, indicating that *Lecithaster* is an aberrant genus of the family HEMIURIDAE. A posterior commissure is present in both the metacercaria and in the adult stages.

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RESEARCH NOTE

A NEW BACTERIUM, *CORYNEBACTERIUM LIPOPTENAE*, ASSOCIATED WITH THE LOUSE FLY, *LILOPTENA DEPRESSA* SAY

Microscopic examinations of the crushed abdomens of specimens of the louse fly, *Lipoptena depressa* (identification confirmed by Mr. Glen M. Kohls) collected from deer in the vicinity of Hamilton, Montana, showed large numbers of a microorganism which took the red dye, as do rickettsiae, when stained by the Macchiavello method. The organisms appeared as small rods which occurred singly and in small masses. Every insect examined out of one lot of 10 harbored this microorganism which appeared to occur chiefly in the intestinal tract. No other microbial forms were encountered in these examinations.

The abdominal viscera of several specimens were inoculated into 6-day, fertile eggs. Smears from these eggs showed from moderate to few or no organisms, but transfer of triturated egg membranes to other fertile eggs resulted in abundant growth. Serial passage by means of the extraembryonic egg fluids was easily maintained through 10 transfers made at approximately 10-day intervals. The organism occurred most abundantly in the egg fluids and moderately in the yolk sac membrane.

From the egg, the organism was successfully transferred to Noguchi's leptospira medium (containing carbohydrates and minced rabbit kidney). From the latter transfers were made to glucose and sucrose agar plates, pin-point colonies resulting. The strains have since been maintained on glucose serum agar slants.

The organism is described as a new species with the following morphologic and cultural characteristics:

Corynebacterium lipoptenae n. sp.

Rods, averaging 0.8 by 4.0 microns, occurring singly and in small masses. Polar and bipolar staining in shorter forms; larger forms may be barred and slightly club-shaped. Many forms stain solidly. Non-motile. Gram-positive. No liquefaction of gelatin. Tiny, gray colonies on dextrose agar. Edges may be slightly curled, rhizoid, and ameboid. Very thin, transparent, gray, more or less filiform growth on agar slant. Rate of growth slow. Broth clear with marked sediment. No change in litmus milk. Starch not hydrolyzed. No visible growth on potato. In ordinary sugar broths, does not ferment dextrose, lactose, sucrose, maltose, mannitol, galactose, arabinose, xylose, dextrin, salicin, raffinose, trehalose, inulin, dulcitol, glycerol, inositol, adonitol, rhamnose, and mannose. Indole not formed. Nitrites not produced from nitrates. Hydrogen sulfide not produced. Aerobe (facultative). Not pathogenic for guinea pigs, rabbits, or mice.

The association of this bacterium (a symbiote?) with *L. depressa* is reported here since it may be similar to that between *Rickettsia melophagi* and the sheep "tick," *Melophagus ovinus*, which belongs to the same family (Hippoboscidae) of insects.—EDWARD A. STEINHAUS, Associate Bacteriologist, U. S. Public Health Service. From the Rocky Mountain Laboratory, Hamilton, Montana, Division of Infectious Diseases, National Institute of Health.

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THE DEVELOPMENT OF THE LARVAL STAGES OF *PLAGIORCHIS MURIS* TANABE, 1922, IN THE FIRST INTERMEDIATE HOST¹

W. W. CORT AND LOUIS OLIVIER²

During the summer of 1941 we undertook a study at the University of Michigan Biological Station of the development of the larval stages of *Plagiorchis muris* in natural infections in the common beach snail, *Stagnicola emarginata angulata* (Sowerby). Very little is known of the mother sporocyst or of the development of the daughter sporocysts of the PLAGIORCHIOIDEA. *P. muris* was chosen for our first study on this group because its larval stages occur in very high incidence in both juveniles and adults of *S. emarginata angulata* in convenient locations near the Biological Station.

Information on the life cycle of *P. muris* in the United States is available in the literature from the work of McMullen (1937a). He studied the mature daughter sporocysts and cercariae and noted that precocious development of the metacercariae frequently occurred inside the sporocysts (McMullen, 1938). Adults were obtained by feeding to pigeons, rats, mice and man (McMullen, 1937b) either precociously developed metacercariae from naturally infected snails or those from experimentally infected insect naiads. Natural infections have been reported in the robin, herring gull, night-hawk, spotted sandpiper, and the muskrat. No experimental infections of the first intermediate hosts have been carried out with this species and nothing has been reported on the mother sporocyst stage, the development of the daughter sporocysts, or the germ cell cycle.

To obtain the early developmental stages of the sporocysts of *P. muris*, we examined between July 21 and September 4, 1941, several hundred juveniles and some adults of the common beach snail, *S. e. angulata* from a beach on Burt Lake (the Burt Lake area, Cort et al, 1937) where the cercariae of this species had been found for several years in very high incidence. The other larval trematodes in this host in this area were chiefly strigeids, schistosomes, notocotylids, and echinostomes. The early developmental stages of members of these groups can be distinguished without the slightest difficulty from those of the plagiorchids. The only other larval stages of plagiorchids that have been found in a large series of examinations of this host in this area have belonged to *P. proximus* Barker, 1915, *P. micro-*

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¹ A contribution from the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University. This is the third of a series of studies on the development of the sporocysts and the germ cell cycle of digenetic trematodes. The earlier investigations dealt with the strigeids (Cort and Olivier, 1941) and a schistosome (Cort and Olivier, 1943).

² Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md.

canthos Macy, 1931, and *Cercaria talboti* (McMullen, 1938). The last two were so very rare that they can be disregarded. The mother sporocysts and the early stages of the daughters of *P. muris* can be distinguished from those of *P. proximus*, which will be covered in a later paper. We can, therefore, be practically certain that the stages described in this paper belong to *P. muris*.

The snails to be examined were carefully pulled out of their shells, and teased apart with very sharp needles under a binocular dissecting microscope. Any structures suspected of being mother sporocysts or immature daughter sporocysts of plagiorchids were carefully freed from the tissues and removed to a slide for study. The preparations were made either with tap water or 0.5 per cent saline, and were studied with the compound microscope under magnifications up to almost 1000. By examining large numbers of snails we were able to secure and study a considerable series of the developmental stages of the sporocysts of *P. muris*.

MOTHER SPOROCTYST STAGE

In the examinations of the snails we looked carefully through all the tissues and especially along the whole length of the intestine to see if we could find mother sporocysts of *P. muris*. Since we had no idea of what the mother sporocyst would be like in this species, we could only study any suspicious looking structures that were found. On July 25, we found in two juvenile snails peculiar masses attached to the outer wall of the intestine. They were composed almost entirely of large numbers of what appeared to be daughter sporocyst embryos. During the course of our examinations about 20 such masses were found and further studies convinced us that they represented late stages of mother sporocysts of *P. muris*. When these mother sporocysts were found in the snails no other stages were present except in a very few cases which were obviously double infections.

These mother sporocysts were round or oval with the greatest diameter varying from 0.5 to 1.7 mm (Figs. 1 and 2); their thickness was distinctly less than their width, and they seemed to be roughly lens-shaped. They were solid, discrete structures with a regular outline and adhered firmly to the outside of the intestine, being attached usually by an edge but sometimes by one of their broad surfaces. They were found both on the first part of the intestine before it reaches the digestive gland and on the last part which passes from the digestive gland to the anus. When present on the middle portion of the intestine, which is imbedded in the surface of the digestive gland, they were imbedded in the glandular tissue and were often broken in pulling the intestine free. Some of these mother sporocysts were freed unbroken, since the daughter sporocyst embryos, of which they are composed, were tightly held together. In surface view, the masses appeared to be made up of numerous compartments (Figs. 1 and 2), which on closer inspection turned out to be closely packed daughter sporocyst embryos. It was estimated that each of the mother sporocysts was composed of from 300 to 500 of these embryos. All those in the same mother were at about the same stage of development; in the smallest they were round or oval, and in the largest, they were much larger, elongate sacs, which showed considerable mobility when freed from the mass. No orderly arrangement of the embryos could be seen in the mother sporocyst masses. The daughter sporocyst embryos of all sizes (Figs. 3-8) were covered by an outer coat of irregular cells; and, in fact, the whole matrix of the mother sporocyst was composed of this one

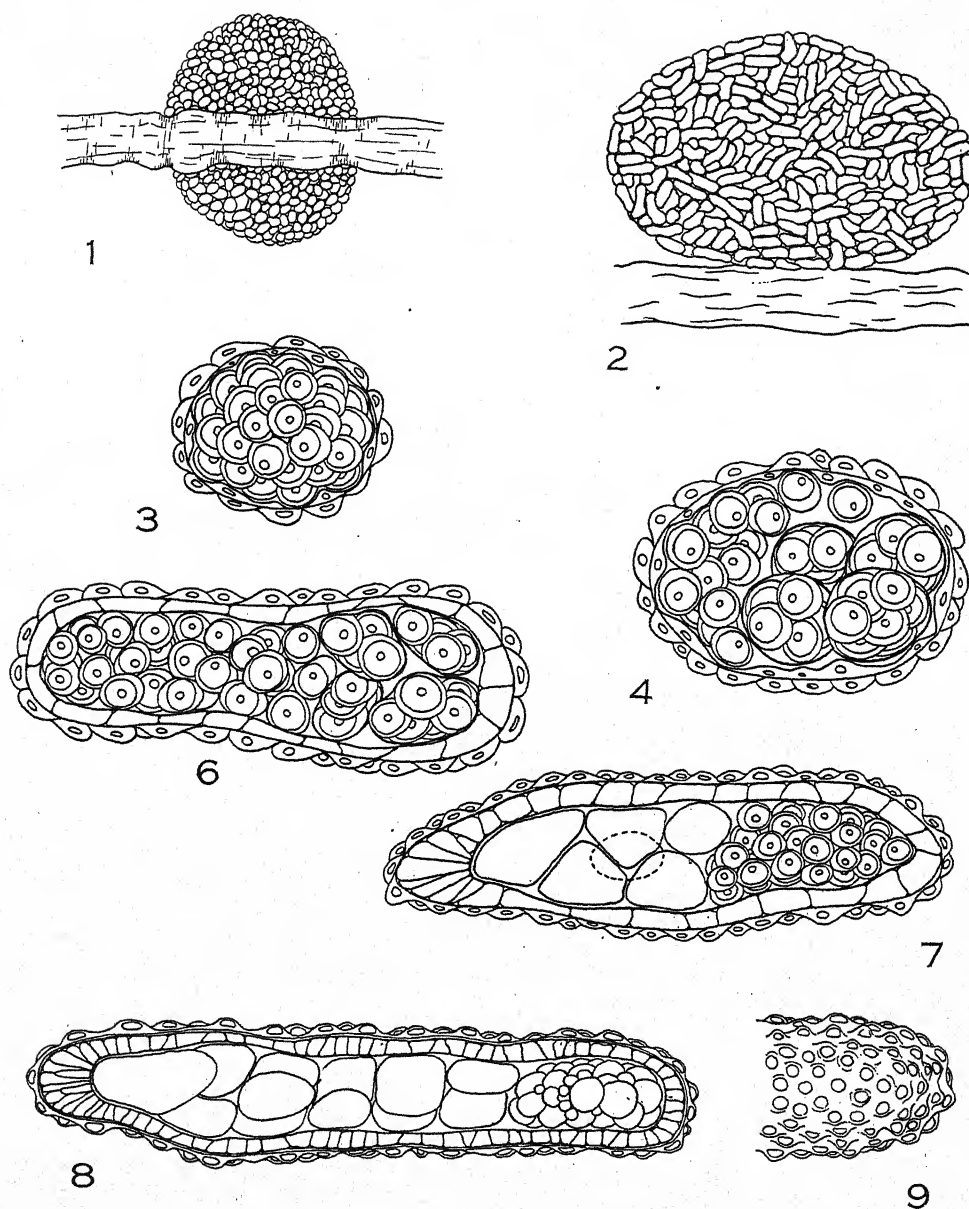


FIG. 1. Mother sporocyst mass attached to intestine of snail. Greatest dimension 0.5 mm. One of youngest stages found with daughter sporocyst embryos very immature (at about the stages shown in Figs. 3 and 4).

FIG. 2. Mother sporocyst mass attached to intestine of snail. Length 1.7 mm. Daughter sporocyst embryos at about the stage of Fig. 8.

FIGS. 3 and 4. Very young embryos separated from the mass of the youngest mother sporocyst of our series. Length, Fig. 3, 0.3 mm; Fig. 4 to scale.

FIGS. 6 and 7. Stages in development of daughter sporocyst embryos from mother sporocyst masses. Length, Fig. 6, 0.1 mm; Fig. 7, 0.13 mm.

FIG. 8. Daughter sporocyst embryo about ready to escape from mother. Note the fully developed outer coat, the paletot, and the discrete germ mass. Length, 0.2 mm.

FIG. 9. Surface view showing the configuration of the paletot.

type of cell. This outer coat was also present in all the later stages of the daughter sporocysts of this species, including those that were mature or old. It appears to be the same as the outer layer described by some of the earliest workers on larval trematodes on the sporocysts of certain species of xiphidiocercariae. It has also been reported more recently by several workers in descriptions of daughter sporocysts of this group. This outer layer was very aptly called the paletot (overcoat) by Bieringer (1885).

STAGES OF DEVELOPMENT OF THE DAUGHTER SPOROCYST EMBRYOS

The youngest daughter sporocyst embryos removed from mother sporocysts, which were at about the stage of Fig. 1, were round or slightly oval and measured from about 0.030 to 0.040 mm in diameter (Fig. 3). The true sporocyst wall at this stage consisted of a thin membrane composed of flattened cells. The contents consisted of a mass of separate germ cells, which were so closely crowded together that they looked almost like the morula stage of an embryo. In the same mother sporocysts with these smallest embryos there were also slightly larger, distinctly oval daughter sporocyst embryos about 0.050 to 0.070 mm in diameter (Fig. 4). In these the wall was slightly thicker and its cellular elements more distinct. The germinal material was not as tightly packed and two to four cercarial embryos at a very early stage of development were present at one end. In two somewhat older mother sporocysts the daughter sporocyst embryos varied from about 0.050 to 0.115 mm in length and from about 0.034 to 0.057 mm in width. The largest of them were distinctly more elongate (Fig. 6). The true wall of the sporocyst was much thicker and was composed of a single layer of large cells. The number of cercarial embryos that had started to develop had increased to about 5 to 7. They were located at one end of the body cavity and the rest of the cavity was filled with single germ cells.

At a somewhat later stage (Fig. 7) the number of developing embryos varied from 6 to 10, and the largest of them were much further along in development so that their individual cells could not be so clearly distinguished. In a mother sporocyst, containing many embryos at about this stage, they measured 0.064 to 0.160 mm in length and 0.040 to 0.061 mm in width. In another somewhat older mother sporocyst all the daughters were larger and distinctly elongate, varying in length from 0.140 to 0.160 mm. In these daughters the sporocyst wall had increased in thickness especially at the ends, the cells having become very distinct. Each daughter sporocyst contained 6 to 8 rather well developed cercarial embryos, and some smaller embryos.

In the oldest mother sporocysts observed the daughters (Fig. 8) were elongate, very mobile, when freed, and appeared capable of migration. In one such mother they varied from 0.120 to 0.200 mm in length and from 0.035 to 0.048 mm in width. In others, some of them were considerably larger. They were very mobile and could extend and contract their bodies, causing the contents of the well defined body cavity to move freely back and forth. The paletot was entirely separated from the few matrix cells that still remained in the small spaces between the crowded daughter sporocysts for the mother sporocyst had become almost entirely a mass of daughter sporocysts. The cells of the paletot were crowded close together and appeared on surface view of the sporocysts as elevations or bosses (Fig. 9). The true body wall of the daughter sporocyst had become much thicker and its layer of cells more

prominent, especially at the ends where distinct thickenings occurred. In one end of the body cavity usually the more mobile anterior end, there was present a discrete, definitely organized germ mass (Fig. 8) made up of multicellular units of different sizes, and single cells. Besides a few very small embryos near the germ mass, the body cavity contained several larger cercarial embryos.

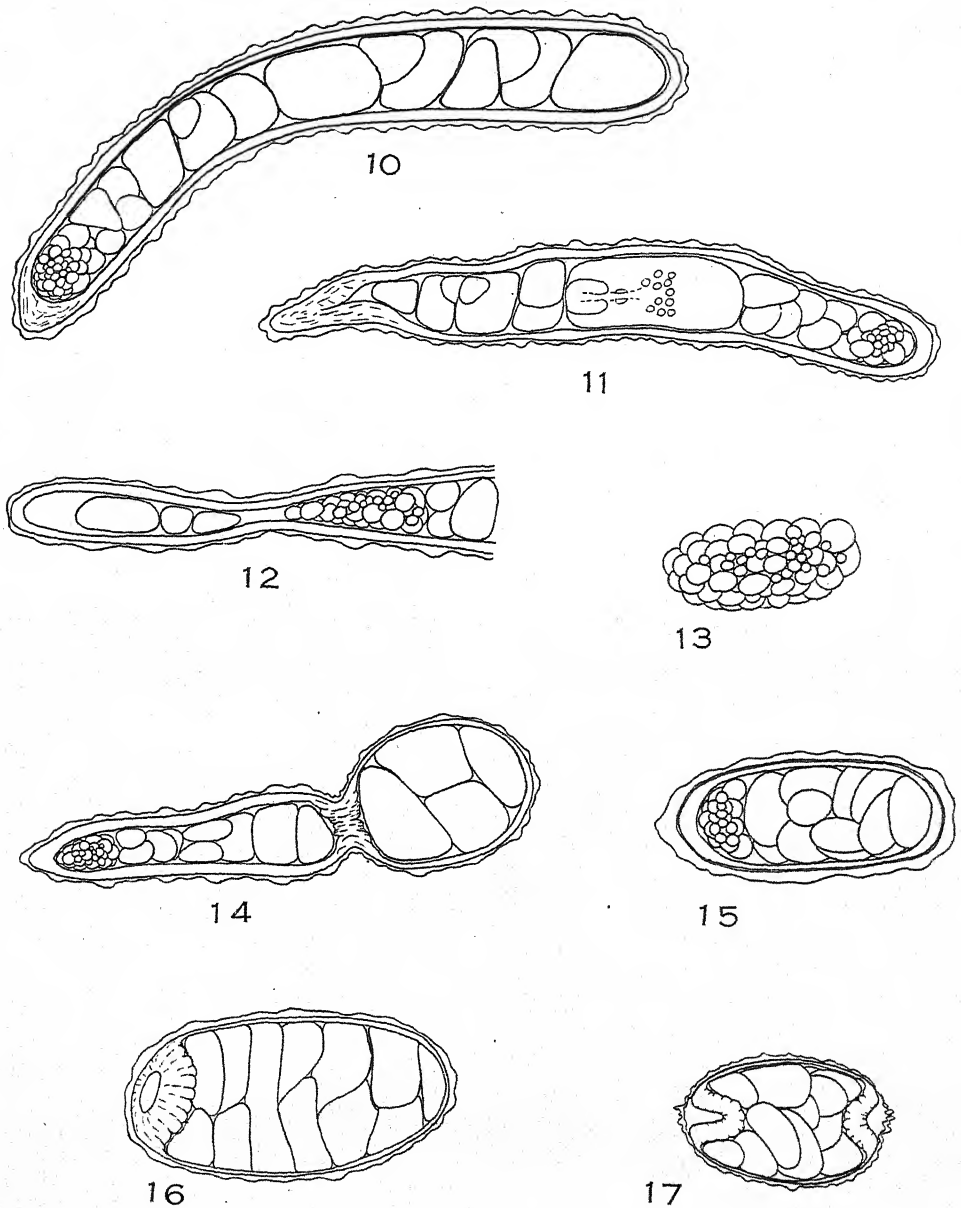
In one immature daughter sporocyst it was possible to make out the excretory system, which consisted of two pairs of flame cells attached to two entirely separate tubules.

MIGRATING DAUGHTER SPOROCYSTS (FIGS. 10 AND 11)

The first clue to the further development of the daughter sporocysts of *P. muris* came from the finding of sporocysts, exactly like the oldest in the mother sporocyst masses, scattered throughout the snail tissues. Some were found on the wall of the stomach and intestine, along the blood vessels leading from the heart, and along the ducts and vessels that pass out of the digestive gland; but most of them were located in the digestive gland along the ducts and vessels that extend to every part of this organ. In two cases, large numbers of these migrating daughter sporocysts were found in the digestive gland and on the viscera while the remnant of a mother sporocyst was still adhering to the intestine. In other infections no trace of a mother sporocyst remained and numerous daughters were apparently migrating through the snail. In still others, only a part of the sporocysts observed were in the migrating stage and the rest were inactive. It appears, therefore, that when the daughter sporocysts break away from mothers located on the parts of the intestine in front of the digestive gland, they migrate extensively along the blood vessels, the reproductive ducts, that come from the hermaphroditic organ inside the digestive gland, and along the ducts from the digestive gland itself. These daughter sporocysts seem to be very effective migrators on account of their great power of extension and contraction and the roughness of their outer surfaces produced by the projecting cells of the paletot. They seem, also, to carry out the complete migration in a comparatively short time, since as already noted, they may reach all parts of the digestive gland before all have escaped from the mother sporocyst mass.

The migrating daughter sporocysts of *P. muris* were very mobile, being able to extend themselves to at least twice their normal length (Fig. 12). As stated above, it is estimated that between 300 and 500 of them are produced by each mother sporocyst, and in one infection an incomplete count gave 265. They varied considerably in size; in one infection they had a range from 0.192 mm by 0.077 mm to 0.512 mm by 0.070 mm. Migrating daughters measured from other infections also fell within this range.

In structure, the migrating daughter sporocysts (Figs. 10 and 11) have advanced but little beyond the largest stages obtained from the oldest mother sporocysts. The body cavity contained 12 to 15 fairly well developed cercarial embryos, some that were still very small, and the germ mass. The largest embryo, in which sometimes the suckers and other structures of the cercaria could be made out (Fig. 11), was usually located at about the middle of the sporocyst and was sometimes as much as 0.1 mm in length. The germ mass was usually at the more attenuated anterior end of the sporocyst, sometimes next to the end, but frequently separated from it by one or a few embryos. In a few cases, it was located at the posterior end or near the



FIGS. 10 and 11. Daughter sporocysts in the migrating stage. Length, Fig. 10, 0.5 mm; Fig. 11, 0.44 mm.

FIG. 12. Attenuated anterior end of daughter sporocyst in migrating stage.

FIG. 13. Germ mass from migrating daughter sporocyst. Length, 0.075 mm.

FIG. 14. Daughter sporocyst in transition from migrating to fixed stage.

FIGS. 15, 16 and 17. Daughter sporocysts just after attachment to snail's tissues. Note shortening and thickening and growth areas at ends. All about 0.2 mm in length.

middle of the body cavity. The germ mass had increased in size and was sometimes as much as 0.075 mm in length. It appeared as a definite, discrete structure like the germ masses of the strigeids (Cort and Olivier, 1941), moved as a unit, and was surrounded by its own membrane (Figs. 8, 10, 11, 12, and 13). It was composed of both single and many-celled components, the largest of which had the same size and structure as the smallest free cercarial embryos. It is suggested that, as in the strigeids, the breaking off of the largest components of the germ mass continually adds to the number of free embryos inside the sporocyst.

When the mother sporocysts were located on the part of the intestine imbedded in the digestive gland, migration of the daughters appeared to be distinctly limited. In some of these cases it appeared that part of the daughter sporocysts had migrated away from the mother into all parts of the gland and that others had developed in a mass at the position of the mother sporocyst. In other cases only a few of the sporocysts appeared to have migrated and in a few cases all had developed in a single mass without leaving the mother. In fact, in one case mature daughter sporocysts were found in a large mass between the foot and gland attached to the intestine. We interpreted this also as a case of development without migration from the mother sporocyst.

STAGES OF SPOROCYST DEVELOPMENT AFTER MIGRATION

In some immature infections in which migrating daughter sporocysts were present, other sporocysts of an entirely different type were found. They were non-motile and were firmly attached along the ducts and vessels in the digestive gland, in front of this organ in the region near the heart, and on the walls of the stomach and intestine. They varied greatly in shape and some were very irregular. They appeared to be firmly attached along their long axis to the hosts' tissue, but just how this attachment was effected was not clear. In some infections all the sporocysts appeared to have just stopped migrating and become attached.

Some of these immature attached daughter sporocysts were oval and considerably shorter and thicker than the migrating stage (Figs. 15, 16, and 17). Fig. 14 appears to show a transition stage from the shape of the migrating sporocysts. In many of them the true sporocyst wall at either or both ends showed thickened areas with the cellular layer greatly enlarged (Figs. 16 and 17). At the centers of these thickened areas there were usually invaginations which gave them a sucker-like appearance. These sporocysts were crowded with cercarial embryos which were so tightly packed that no space whatever was left between them and their shapes were modified by contact with each other (Fig. 16). The largest of the cercarial embryos showed the suckers and other structures of the body, but were still very immature.

Also, in these immature infections some sporocysts were present, which bore projections that appeared to arise from the centers of the thickened areas at the ends. Their projections were sometimes small and bulb-like (Fig. 18), but frequently larger and either round or elongate (Figs. 19 and 20). Some of them were made up entirely of sporocyst wall (Figs. 18, 19 and 20), but in many cases the body cavity containing cercarial embryos extended into them (Figs. 22 and 23). In some of these immature sporocysts the projections were round, crowded with embryos and almost the same size as the original sporocyst (Figs. 21 and 25), giving the appearance of fission or budding. Frequently, the two parts filled with embryos were separated by a tube of varying length containing no embryos (Fig.

24). In some cases even, sporocysts were divided into three approximately equal divisions (Fig. 26). In fact, in immature infections of this type, in which none of the cercarial embryos were even approaching maturity, sporocysts of a wide variety of shapes, some of which were very bizarre, were found (Fig. 31). In some of the larger of the irregularly shaped sporocysts the cercarial embryos showed the beginnings of a tail. Sometimes, in an infection there would be found some of the irregularly shaped sporocysts of the type just described and others that were larger and more regular in shape with some cercariae approaching maturity.

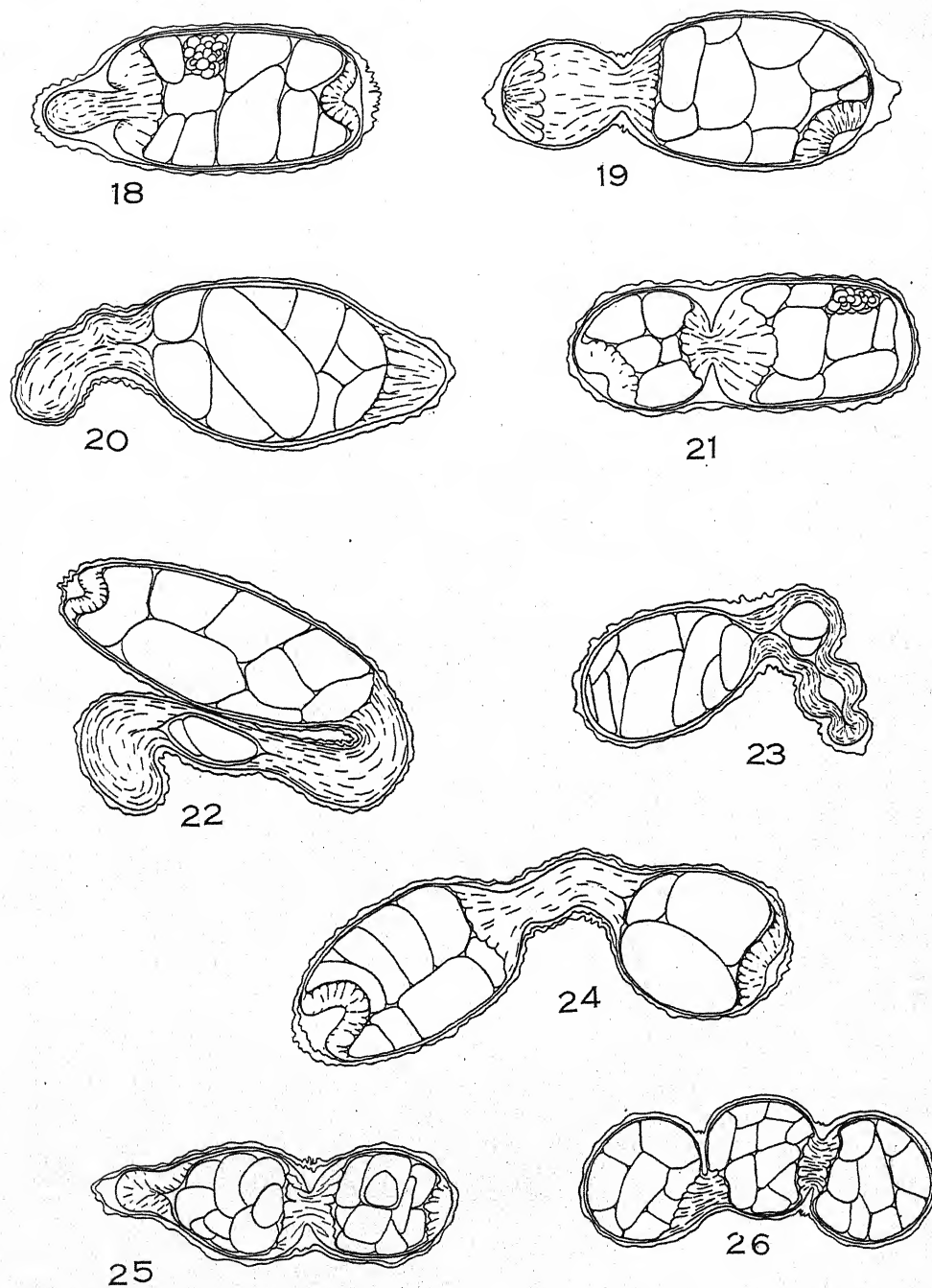
Except where it was torn off by manipulation, each sporocyst was surrounded by the outer layer originally formed of mother sporocyst cells, the paletot, which in places was not closely attached to the true sporocyst wall (Figs. 18, 21 and 25). In a few cases these developing stages were found in sporocysts that had never migrated from the mother, especially when the mother sporocysts were imbedded in the digestive gland.

Only a few infections were found in which the daughter sporocysts had developed beyond the small irregular stage just described, and yet had not begun to produce mature cercariae. In one such infection in an adult snail the oldest cercariae had well developed tails, but were still immature. The sporocysts in this case varied in size from 0.26 by 0.06 mm to 0.68 by 0.12 mm, with most of them under 0.40 mm in length. They showed a variety of shapes (Fig. 32) but were much more regular than those shown in Fig. 31, and were filled with embryos. It can be seen by comparing the outline drawings of sporocysts from this infection (Fig. 32) with those shown in Fig. 31, that they were no longer than those of the earlier stage, but differed chiefly in having a greater width with no projections or narrower parts that were not filled with embryos.

In the infection just discussed we examined almost every sporocyst, but could not find a single case in which two or more sporocysts were enclosed in a single paletot. In fact, after looking exhaustively in a large series of infections, both those containing only immature cercariae and those with mature cercariae, we were able to find only a very few cases which suggested that two or more separate sporocysts were enclosed in a single paletot; and only in the three cases shown in the drawings (Figs. 27, 28, and 29) could we be reasonably certain that the sporocysts inside the same paletot were definitely separated.

MATURE SPOROCASTS

Large numbers of young mature infections of *P. muris* were found, especially in the juvenile snails. They were characterized by the presence of small numbers of mature cercariae. In most cases the sporocysts were somewhat larger in size than in infections such as that just described in which none of the cercariae were yet mature. However, in a few cases where mature cercariae were present the size was somewhat less, as for example, one in which the sporocyst size varied from 0.33 by 0.12 mm to 0.61 by 0.19 mm. The sporocysts at this stage were plump and filled with embryos, and the few mature cercariae that were present moved actively. Careful examination disclosed the presence in each sporocyst of a single germ mass as well as numbers of small cercarial embryos in different stages of development. The paletot had become a thicker, firmer membrane and occasionally showed some orange pigmentation and the true sporocyst wall was relatively thinner than in the earlier



FIGS. 18-26. Very immature daughter sporocysts in growth stages. Note areas of growth and variety of shapes assumed. Length of Fig. 18 is 0.26 mm. Figs. 18 to 24 drawn to scale.

stages. The majority of the sporocysts at this stage were quite regular in shape, but a number were distinctly irregular (Fig. 33). The end from which the cercariae escaped was more pointed than the other, but the birth pore could not be made out. Young mature sporocysts varied greatly in size, both in the same infection and in different infections. A few were less than 0.5 mm in length and occasionally the largest was over 1 mm. Most, however, were between 0.5 and 0.8 mm in length and from 0.1 to 0.2 mm in their greatest diameter. All but the three largest of the outline drawings of Fig. 33 were made from young mature sporocysts.

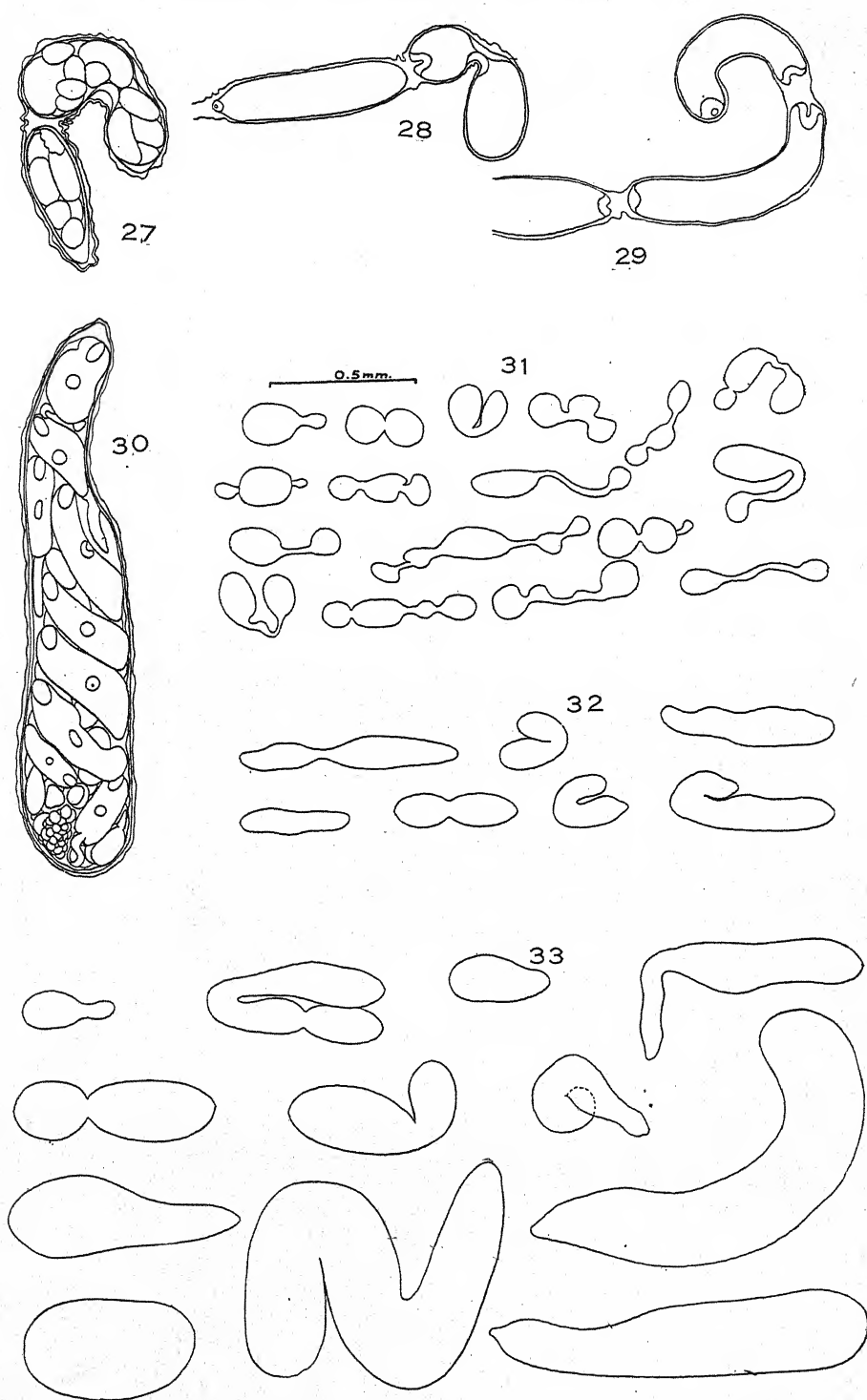
Older daughter sporocysts with larger numbers of mature or almost mature cercariae were larger (Fig. 30). The paletot was still thicker and contained a considerable amount of orange pigment. The number of mature, or almost mature, cercariae in each sporocyst was larger, usually being more than 10. The birth pore was located at the pointed end but could only be seen clearly when cercariae were escaping. Developing embryos at all stages were present and the germ mass appeared unchanged (Fig. 30).

In the oldest infections which were found in old snails in September some of the sporocysts were from 1 mm to 1.6 mm in length. Occasionally, still larger ones were found, the largest measured being almost 3 mm in length and containing about 40 mature or almost mature cercariae. However, in most mature infections including the oldest, the sporocysts averaged under 1 mm in length and in some cases, were less than 0.5 mm. Thus, a considerable variation in size of sporocysts between infections was found, and the variation in a single infection was also large. Certainly, if the sporocyst size for other species of plagiorchids varies as much as that for *P. muris*, measurements such as those given in most descriptions have little validity as specific characters.

Even in the very oldest infections in the old snails examined in September, there was no evidence of the exhaustion of any of the daughter sporocysts. They were especially heavily pigmented and, in some cases, not nearly as crowded with cercariae and embryos as the younger ones. Nevertheless, there were always large numbers of mature cercariae still present and germ masses and very small embryos could still be seen. In fact, in several old infections in which precocious development had occurred, germ masses and small embryos could be found crowded in among the metacercariae.

RELATIONS OF INFECTIONS OF *P. muris* TO THE SNAIL INTERMEDIATE HOST

From the studies of the summer of 1941 we obtained some information that supplemented our earlier data on the relations of infections of the larval stages of *P. muris* to the snail host, *S. emarginata angulata* (cf. Cort, 1941). Infection of the snails must begin to take place in the very youngest juveniles since immature daughter sporocysts were found in very small juveniles and a few young mature infections were present by the first of August. In fact, in our examinations of 404 juveniles collected from the Burt Lake area on July 25, 1941, and examined from that date until about the middle of August, we found 23 (5.7 per cent) infections of *P. muris* with some cercariae fully or almost fully developed. Also, in a collection of juveniles made from the same area on August 2, 1938, five infections were mature or almost mature and from three of these cercariae were escaping. Since juveniles of this snail species apparently do not usually hatch on the beaches in any number



FIGS. 27-29. Drawings showing the three cases in which more than one daughter sporocyst was found inside the paletot.

FIG. 30. Mature daughter sporocyst. Length 1 mm.

FIGS. 31, 32 and 33. Outline drawings at about the same scale of the stages of development of the daughter sporocysts; Fig. 31, earliest growth stage; Fig. 32, stages in which cercariae are not yet mature; Fig. 33, young mature and mature stages.

before July 1 (Cort et al, 1940), it seems evident that the development up to the time that the first cercariae are mature may require only a little over a month. New infections continue to enter the juveniles at least until after the first of August since as many mother sporocyst stages were found in the collection of August 21 as in that of July 25. In the various collections of juveniles that have been made in late August and early September of several different summers from the Burt Lake area a high percentage of infection with *P. muris* was always found. This percentage was slightly greater in the collections of adult snails which developed from this same generation of juveniles which were made in June of the next year, increased in later summer collections of adults, and was at its peak in the collections of late August and early September (Cort, 1941). Also, in the adult snails examined from this area in the summer of 1941 mother sporocysts and early developmental stages of daughter sporocysts of this species were found. In these collections, even those made late in the summer, we could distinguish the older infections that had been carried over the winter from those that had entered the same year, since the sporocysts in them were larger, more heavily pigmented, and contained larger numbers of mature cercariae. As noted above, even the oldest infections found in old snails in early September were still actively producing cercariae and the sporocysts contained the youngest embryos and germ masses. It appears, therefore, that infections of *P. muris* enter the snails of this species throughout both summers of their life. Therefore, the oldest infections found in old snails in early September, which were still actively producing cercariae, must have been more than a year old. It can be suggested, therefore, that these snail hosts acquire infections of this trematode from their youngest juvenile stages until they are well past maturity, and that even those acquired by the youngest juveniles probably continue to produce cercariae until the natural death of their host.

Another striking point was the very small amount of injury produced in the snails by the sporocysts of *P. muris* even in old, heavy infections. We have no evidence of their effect on the reproductive organs; but the digestive gland showed but slight injury compared with that produced by old infections with strigeids, schistosomes, and echinostomes. This appears to be due to the fact that the sporocysts are chiefly attached to the ducts and vessels of the gland and are immobile except in the migrating stage. Also, there is no evidence that infected snails are killed by infections of *P. muris* even over the winter, since as noted above, there is a steady increase in the incidence of this parasite all through the life of the snails. On the other hand, striking reductions of incidence of infection of certain echinostomes and strigeids in older as compared with younger snails have been interpreted as showing a differential death rate of the infected snails (Cort et al, 1939; Cort, 1941); also, in infections of these forms considerable damage to the digestive gland of the host is apparent.

DISCUSSION

The most interesting points brought out in the studies of the development of the larval stages of *P. muris* in naturally infected snails have been the following: (1) the finding of mother sporocysts which are entirely different from any previously described for other trematodes; (2) the discovery that the outer membrane, the paletot, of the daughter sporocysts is derived from the wall of the mother sporocyst; (3) the discovery of the method of migration of the young daughter sporocysts and

the description of the migrating stage; (4) the finding of immature sporocysts of irregular shapes with projections formed from areas of growth at their ends; (5) the demonstration of a discrete germ mass in the young daughter sporocyst which persists throughout its whole life; and (6) the tracing of the seasonal cycle of infection in relation to the life cycle of the snail host.

Although in the superfamily *PLAGIORCHIOIDEA*, as defined by McMullen (1937c) to include all forms with true xiphidiocercariae, about fifty life cycles have been worked out to the extent of connecting cercariae with adults, surprisingly little is known of the early stages of development in the snail intermediate host. In only a few of the known life cycles has the intermediate host been infected experimentally and we have found descriptions of mother sporocysts for only four species, all after experimental infection. Three of these belong to one group, the *RENIFERINAE* (Talbot, 1933; Byrd, 1935; Walker, 1939) and the other is *Plagitura parva* (Stunkard, 1936). In all these cases the mother sporocysts are described as thin-walled sacs in which daughter sporocysts develop and from which they later escape and migrate to the position where they continue their development. The outer coat or paletot is not shown for the daughter sporocysts of these species.

The mother sporocyst stages of *P. muris* described above are entirely different from any of those previously described for the *PLAGIORCHIOIDEA*. They appear to represent only relatively late phases of mother sporocyst development. Therefore, we can only make certain very tentative suggestions in regard to their development. In the first place, it seems practically certain from the position of these mother sporocysts on the outside of the intestine that infection of the intermediate host is by ingestion of embryonated eggs. Another point worth stressing is that the germinal material of the early mother sporocyst of this species must have been so organized that all the embryos started to develop at about the same time. Only in this way can the fact be explained that all the daughter sporocyst embryos in any mother sporocyst are in about the same stage of development, and escape from the mother at about the same time. In most other mother sporocysts that have been described the evidence is clear that daughter sporocysts are produced in series over a period of a number of days or even weeks.

It also seems difficult to fit in the behavior and position of the irregular matrix cells of the mother sporocysts of *P. muris* with anything previously known about the development of this stage in other trematodes. These cells form an outer layer around the daughter sporocyst embryos that persists throughout their further development. Such an outer layer (paletot) in daughter sporocysts of certain xiphidiocercariae seems to have been first described by Steenstrup (1842) for the sporocysts of *Cercaria armata*. Moulinie (1856), de Filippi (1857) and Wagener (1857) also saw it on immature sporocysts in infections not far enough along to be identified. Biehringer (1885) and Schwartz (1886) also reported the presence of this layer on the sporocysts of *C. armata* and clearly showed its division into separate cells. The origin of this layer was considered by several authors. De Filippi concluded that it was the remains of a mother sporocyst that had produced a single daughter within itself. Leuckart (1863) suggested that it did not belong to the sporocysts at all but was formed from the host's tissue. Biehringer (1885) accepted this view and presented evidence which he thought demonstrated that the paletot was formed from the cells of the snail's blood which attached themselves to the outside of the

sporocyst and spread themselves out into an epithelial layer. The views of this author on the origin of the paletot were accepted by Leuckart (1889, p. 107) and Braun (Pagenstecher and Braun, 1879-1893, p. 811).

More recent references to the paletot are surprisingly few. Dubois (1929, p. 6) showed what is probably this layer in a cross-section of the wall of the sporocyst of a xiphidiocercaria (*Cercaria helvetica* V), and Harper (1929) described it for the sporocysts of his *Cercaria* X. Brooks (1930) also described an outer "sheath composed of epithelial cells" around the sporocysts of three xiphidiocercaria species. The only other recent reference we have been able to find to the paletot is in the monograph of Wesenberg-Lund (1934). This author mentioned the presence of a "yellow paletot" on the sporocysts of *Haplometra cylindracea* which he found in *Lymnaea stagnalis*. He also described it for the sporocysts of *Cercaria cellulosa* as a very thick layer consisting of flattened cells (Pl. XIV, Fig. 8). In this case cercariae were found between this layer and the outer wall of the sporocyst. The small number of recent references to the paletot is perhaps due to the failure of most authors who describe xiphidiocercariae to pay any attention whatever to the structure of the sporocysts.

We have been unable to find in the literature any descriptions of immature sporocysts of xiphidiocercariae that resemble the migrating stage of *P. muris*. As mentioned above we concluded from our observations that when the mother sporocyst masses are located on the parts of the intestine in front of the digestive gland, the daughter sporocysts migrate widely and become attached chiefly along the ducts and vessels, both between the foot and the gland and within the digestive gland itself. In fact, when the ducts and vessels in the digestive gland were carefully pulled free from the parenchyma practically all the sporocysts present in the gland would remain adhering to them. On the other hand, when the mother sporocysts were located on the surface of the digestive gland migration was much more limited.

When we first found infections containing the small irregular sporocysts of the type shown in Figs. 18 to 26 and 31, we had great difficulty in fitting them into the scheme of development. It was evident from the fact that the cercarial embryos in them were still very immature, that they represented stages just beyond the migrating daughter sporocysts. At first we were inclined to the opinion that they represented a phase of division by budding and fission. It seemed evident that if the irregular, immature sporocysts of *P. muris* were really in a stage of division, the next stages would show numerous cases of completely separated sporocysts within a single paletot. Exhaustive search in which large numbers of sporocysts of different ages were observed in a variety of different infections gave us only the three cases that have been figured (Figs. 27, 28, and 29) in which more than one sporocyst was enclosed by a single paletot. Also, in these examinations nothing else was found that suggested that there had been fission or budding. Furthermore, each sporocyst contained only one germ mass exactly like that found in the migrating stage. It would be very difficult to postulate a method for providing new germ masses for sporocysts produced by budding or fission. We were forced, therefore, to the view that in the usual course of the development of the larval stages of *P. muris* only one mature daughter sporocyst is formed from each embryo of the mother sporocyst.

Later, we became convinced that these irregularly shaped immature sporocysts are really undergoing changes by which the size is increased to make room for the

rapidly increasing embryonic material. According to this view, soon after the migrating stages reach suitable locations they cease movement and become attached to the hosts' tissues. Then the ends of the sporocysts become regions of growth and form projections. The lumina of the projections remain connected with the main body cavity of the sporocysts and embryos are gradually pushed into them. As the projections grow larger they are occupied by more and more cercarial embryos and eventually fill out to the same diameter as the original sporocyst. In addition, new projections may in turn grow out from growth areas at their ends. As larger numbers of new embryos are produced from the germ masses and as those already present increase in size the irregular shaped sporocysts are filled out and become more regular in shape, although still showing some indications of the shapes they had assumed during the growth period (Fig. 32). In fact, some trace of these unusual shapes may still appear in mature sporocysts (Fig. 33). This early growth may, therefore, be considered as a combination of an increase in size of the sporocysts by the growth of projections from the growth areas at the ends and a rapid increase in the amount of germinal material, which crowds into all available space.

It appears that the time from the migration of the daughter sporocysts until the first cercariae become mature is very short, perhaps only a few days. Evidence for this is that the oldest cercarial embryos in the migrating sporocysts are surprisingly large and well developed. Also, in the stages just after migration they soon show the development of the tail and body structures. In several infections, a few mature cercariae were present in some of the sporocysts while others were in the stages showing projections from the growth areas of the wall. In addition, only a very few infections were found in all our examinations in which all the cercariae were still immature and all the sporocysts had developed beyond the early irregular stages of growth. Such rapid growth and development starting immediately after the migration is probably associated with a greatly improved food supply for the daughter sporocysts in their new location as compared with that obtained while still crowded together in the mother sporocyst mass.

The method of growth of the daughter sporocysts of *P. muris* is so peculiar that it seems almost inconceivable that similar stages should not have been frequently seen if this type of development is at all common in the plagiurchiids. The thickenings at the ends of the earliest stages after attachment, the formation of projections from these ends and the bizarre shapes that are assumed are all very striking and should have attracted the attention of observers. We have found, however, only a few scattered observations that suggest a similar development. Among the earliest workers, de Filippi (1857, Pl. I, Fig. VIII) showed a young sporocyst inside its paletot divided into two parts by a narrow constriction. "Suckers" at the ends of immature daughter sporocysts of this group have been reported from time to time and the figures of Biehinger (1885, Tab. 1, Figs. 1, 11, 13, and 14) show that these so-called suckers for *C. armata* are merely growth areas with invaginations at their centers like those found at the ends of the very immature daughter sporocysts of *P. muris*. This author (Fig. 12) also showed a slight protrusion at the center of the growth area of one of these sporocysts which resembles our figure 18. Daughter sporocysts with projections or with irregular shapes have also been noted (Looss, 1896, Pl. XVI, Fig. 178; Harper, 1929). In fact, such findings have been, perhaps,

the chief basis for the views held by certain authors that the daughter sporocysts of the xiphidiocercariae commonly reproduce by fission and budding (Wesenberg-Lund, 1934).

There is very little information available on the germ cell cycle of the *PLAGIORCHIOIDEA*. The miracidia in this group are very small and in the few cases where they have been carefully studied were found to contain only a very few germ cells, as was clearly shown by Talbot (1933) and Walker (1939). These authors also studied very immature mother sporocysts and their descriptions indicate an early rapid multiplication of the germ cells to form large numbers of daughter sporocyst embryos. Our observations on *P. muris* add nothing on this stage. We have found nothing in the literature on the germ cell cycle in the development of the daughter sporocysts except the work of Brooks (1930). This author merely stated that "germinal multiplication is by the formation of germ masses." According to his view these germ masses break up into single cells, the "ex-components," each of which forms a cercarial embryo.

Our studies give some information on the germ cell cycle in the daughter sporocysts of *P. muris*. In the youngest embryos we observed there had already been a considerable multiplication of germ cells and from these cells cercarial embryos soon begin to develop. This type of development of new cercarial embryos from individual germ cells seems to continue throughout the early stages. But later a definite, discrete germ mass appears. Perhaps this develops from one of the separate germ cells, while all the others go on to cercarial embryos. Unfortunately, our observations are not at all clear on this point. At any rate, by the time the daughter sporocysts are ready to migrate from the mother each contains a single discrete, rather large germ mass surrounded by its own membrane, and no separate single germ cells are present in the body cavity. The smallest free bodies found at this stage are multicellular cercarial embryos that have the same size and structure as the largest multicellular components of the germ mass. From repeated examinations of the contents of daughter sporocysts at all stages, we conclude that in each a single germ mass persists all through development and that no free germ cells are present.

As noted above, these germ masses are still present in very old daughter sporocysts in old snails that would soon die from old age. Such sporocysts while not as crowded with developing cercariae as the younger ones, still contain numbers of mature and almost mature cercariae and all developmental stages including the very smallest embryos. The conclusion seems inescapable that after the formation of the germ mass, the only method of production of new cercarial embryos is by the splitting off of its largest multicellular components. We interpret this as a special type of polyembryony like that described for the strigeids (Cort and Olivier, 1941).

One of the most striking things in the development of *P. muris* is the long life possible for the daughter sporocysts and the very large number of cercariae that a single infection can produce. In fact, enormous cercarial production seems to be characteristic of the *PLAGIORCHIOIDEA* (Cort, 1922; Dubois, 1929). In the development of *P. muris* in the snail intermediate host the daughter sporocysts that migrate from a single mother sporocyst mass begin to give off mature cercariae at about the same time, perhaps in a month to six weeks after the snail is infected. It seems probable that the production of cercariae continues until the infection is terminated by the death of the snail. Therefore, in an infection that entered a very young juve-

nile snail, cercariae might begin to escape by the end of July and would be produced in increasing numbers until activity ceased with cold weather. As soon as the temperature warmed up sufficiently the next spring, cercarial production would begin again and would last until the natural death of the snail in the late summer or fall. It seems probable that in such infections each daughter sporocyst would produce hundreds of cercariae throughout its long life and that the total number that developed from a single miracidium might reach almost a million. Such an enormous production of cercariae appears to be achieved partly by a considerable multiplication of the few germ cells set aside during the development of the miracidium to form the several hundred daughter sporocysts produced by a single mother. Most important, however, is the reproductive ability of the large germ masses which develop in the daughter sporocysts before they migrate from the mother and which, by a special type of polyembryony, produce a constant stream of cercarial embryos throughout the whole life of the infection.

In addition to this extraordinary production of cercariae, which it seems to share with other plagiurchiids, *P. muris* has other special characteristics that appear to account for its great success as a parasite. Its larval stages in the snail are so located and their habits are such that they do not seem to produce serious injury to their host. Certainly, there is no evidence of an increase in the death rate of the snails due to the presence of this species such as has been found in certain other types of larval trematode infections. In addition, the metacercariae can develop not only in a variety of aquatic insect larvae, but, also, precociously inside the sporocysts in the first intermediate host, making possible infection of animals that feed on these snails. Finally, a lack of specificity in definitive host relations makes possible the development of the adult in a wide variety of both birds and mammals.

SUMMARY

During the summer of 1941 large numbers of juveniles and adults of the common beach snail, *Stagnicola emarginata angulata* (Sowerby) were examined from a beach on the shore of Burt Lake in northern Michigan, where there was known to be a high incidence of the larval stages of *Plagiorchis muris*. A considerable series of late stages of mother sporocysts was found, especially in juvenile snails. They are oval, irregularly disc-shaped masses, 0.5 to 1.7 mm in largest diameter, which are composed almost entirely of daughter sporocyst embryos (about 300 to 500 in each). These mother sporocysts are firmly attached to the outside of the snail's intestine, and may occur anywhere along its whole length. The matrix of the mother surrounding the daughter sporocyst embryos consists of irregular cells which form an outer layer around the daughter sporocyst embryos. This outer layer, the paletot of earlier workers, persists throughout the whole life of the daughter sporocysts. The largest daughter sporocysts still found in the mother sporocyst masses are elongate and mobile, and each contains cercarial embryos of different ages, and a single discrete germ mass. When located on the parts of the intestine between the foot and digestive gland they break out of the mother sporocyst, all at about the same time, and migrate along the ducts and vessels to the walls of various organs and to all parts of the digestive gland of the snail. When the mother sporocysts are on the surface of the gland the migration of the daughters is much more limited and there is a tendency for them to develop without leaving the mother. When the daughter

sporocysts cease migrating they become firmly attached, thicken, and soon appear crowded with embryos. Soon, thickened areas are formed at their ends, which frequently have invaginations at their centers making them look like suckers. From these "areas of growth" protrusions push out. Further growth of these protrusions and their occupation by cercarial embryos produce a variety of irregularly shaped sporocysts, each enclosed in its paletot. Later, with increasing size of the sporocyst wall and rapid growth and production of cercarial embryos, the shapes of the daughter sporocysts become less irregular. Growth in size of the sporocysts continues after the escape of the first mature cercariae, and the paletot becomes pigmented after maturity is reached. The germ mass persists throughout the whole life of the daughter sporocyst, and produces a constant stream of cercarial embryos by a process that has been interpreted as a special type of polyembryony like that found in the strigeids. Even in the very oldest, most heavily pigmented sporocysts from old snails there is no sign of exhaustion of cercarial production. Infections of *P. muris* enter the snail host both in its juvenile and adult stages and probably produce cercariae actively until the death of the host. Because of their position along the ducts and vessels and on account of their inactivity, the daughter sporocysts do not appear to produce serious injury to their host. It is suggested that the great success of *P. muris* as a parasite is due to its extraordinary power of cercarial production, the absence of serious injury to the snail by its larval stages, and the lack of specificity of its metacercarial and adult stages.

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REPORT ON A COLLECTION OF BRANCHIOBELLEIDS

CLARENCE J. GOODNIGHT

The writer has had the opportunity to examine an interesting collection of branchiobdellids which were received from G. K. Goellner of the University of Michigan. In the collection, two new forms were encountered, and several new records of previously described forms have been added to our knowledge of the distribution of this interesting group of worms.

Cambarincola chirocephala Ellis, 1919

Records: Fenton Rearing Ponds, Oakland County, Michigan, Coll. G. K. Goellner; and James River, Gallow, Missouri, Coll. Creaser and Williamson.

Cambarincola macrocephala n. sp.
(Figs. 1 and 2)

Description: *Cambarincola*: Worm cylindrical, head large, expanded so that it is distinctly wider than anterior body segments. Head separated by deep constriction from Segment I. Dorsal lip with four finger-like projections, ventral lip entire. Body segments increasing in width from I to VII, posteriorly decreasing to the terminal sucker. Major annulations of body segments not elevated over minor annulations. Ventral and dorsal jaws similar, appearing as large triangular blocks of chitin terminating in sharp tooth. Without lateral teeth but margin uneven. Width of jaws at base 400 μ . Pharyngeal diverticula four, two dorsal and two ventral. Alimentary canal in medial portion straight, with large sacculum in segments II and III and small sacculations in segments IV, V, VI and VII. Posterior to segment VII, narrows rapidly to anus in dorsal portion of segment X. Testes in pairs in segments V and VI. Vasa deferentia joining atrium in VI; with accessory sperm tube. Ovaries in segment VII. Length of mature worm 2.0 to 4.0 mm.

Locality: Polecat Creek, Northern Teton County, Wyoming, August 16, 1941. Coll. Robert C. Brown.

Host: *Astacus gambellii*.

Holotype specimen: U. S. Nat. Mus. Coll. No. 20598.

This species differs from all known members of *Cambarincola* in the type of jaws.

Cambarincola macrondonta Ellis, 1912

Records: Fenton Rearing Ponds, Oakland County, Michigan, Coll. G. K. Goellner; and State Hatchery Ponds, Pratt, Pratt County, Kansas, October, 1939, Coll. Robert C. Brown.

Cambarincola philadelphica (Leidy, 1851)

Records: Muddy Creek, La Monte, Missouri, September 2, 1930, Coll. E. P. Creaser; and Reed Creek, Wortle County, Virginia, May 17, 1931, Coll. C. L. Hubbs and E. P. Creaser.

Cambarincola vitrea Ellis, 1919

Records: Cheyenne, Wyoming, September 18, 1941, Coll. James C. Simpson; and Fenton Rearing Ponds, Oakland County, Michigan, Coll. G. K. Goellner.

Pterodrilus distichus Moore, 1895

Record: Fenton Rearing Ponds, Oakland County, Michigan, Coll. G. K. Goellner.

Xironodrilus appalachius n. sp.
(Figs. 3 and 4)

Description: *Xironodrilus*: Body rather elongate, and distinctly depressed. Width of head approximately equal to segment I. Body segments increasing regularly in width from segment

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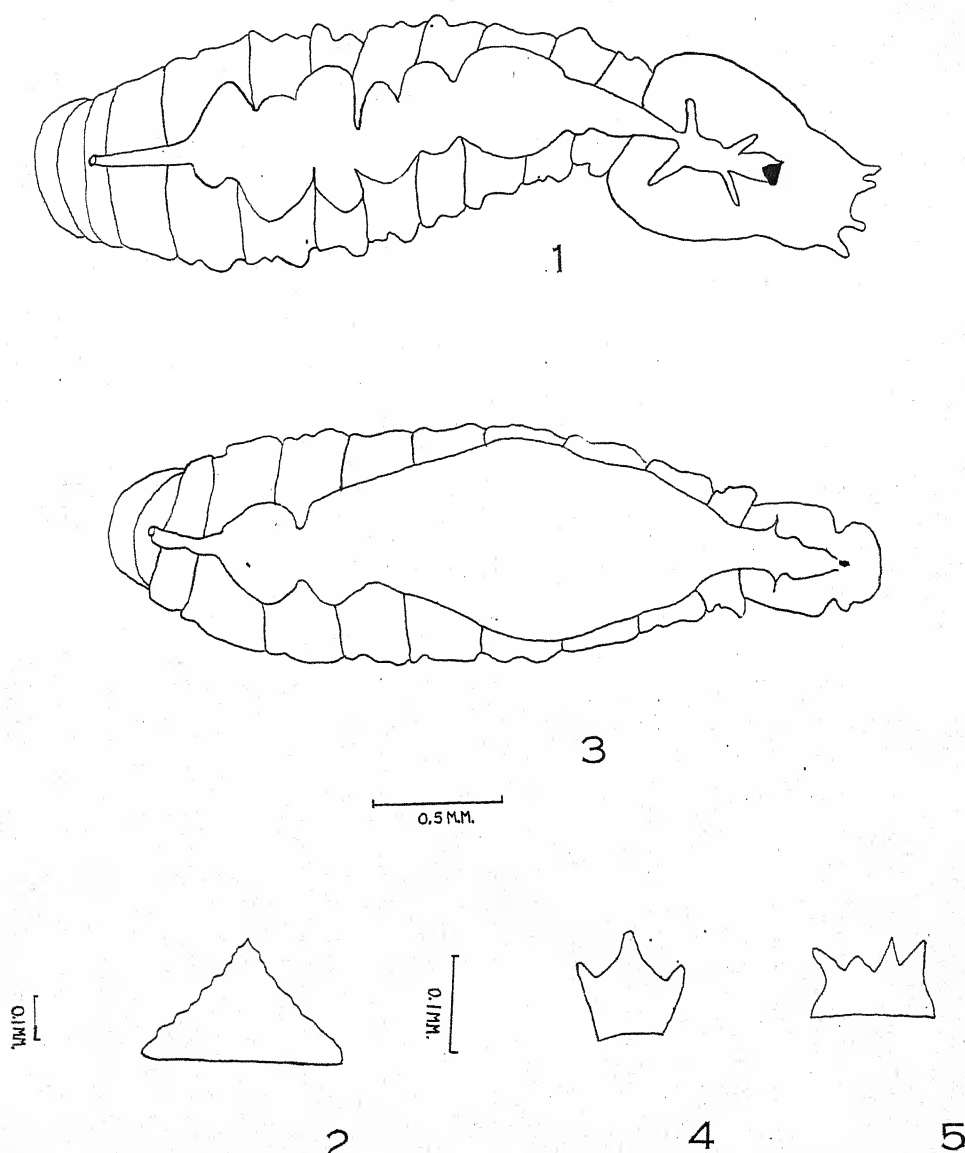


FIG. 1. Dorsal view of *Cambarincola macrocephela* n. sp.
 FIG. 2. Dorsal jaw of *Cambarincola macrocephela* n. sp.
 FIG. 3. Dorsal view of *Xironodrilus appalachius* n. sp.
 FIG. 4. Dorsal jaw of *Xironodrilus appalachius* n. sp.
 FIG. 5. Dorsal jaw of *Xironodrilus dentatus* Goodnight, 1940.

I to VII, posteriorly decreasing to caudal sucker. Small accessory suckers present near lateral margins of ventral surfaces of segments VIII and IX. Segments I to IX distinct, major annulations not elevated over minor elevations. Upper and lower lips entire, with small median emarginations. Tooth formula 3-3. Median tooth longer than lateral teeth. Major pharyngeal diverticula two in number, one dorsal and one ventral. Alimentary canal passing through body near medial axis, somewhat expanded in segment I, strongly sacculated in segments II to V, narrowing in segment VI, somewhat enlarged in segments VII and VIII, narrowing rapidly from segment VIII to anal opening on dorsal surface of segment X. Testes present in segments V and VI, vasa deferentia joining atrium segment VI. No accessory sperm tube present. Spermatheca

in segment V, simple. Each anterior nephridia opening to outside through separate pores on dorsal surface of segment III. Ovaries present in segment VII. Length of mature worm approximately 3 mm. Width of upper and lower jaw at the base 60 μ .

Locality: Clingman's Dome, Great Smoky Mountains National Park, September 9, 1937. Coll. A. R. Cahn.

Host: *Cambarus* sp.

Holotype specimen: U. S. Nat. Mus. Coll. No. 20596.

This species shows its closest affinities to *Xironodrilus pulcherrimus* (Moore, 1894), but differs in having the median tooth longer than the lateral ones, whereas in *pulcherrimus* it is shorter.

Xironodrilus dentatus Goodnight, 1940
(Fig. 5)

Record: James River, Gallow, Missouri, Coll. Creaser and Williamson.

Additional material found for study, leads the writer to believe that this name should be elevated to specific rank instead of subspecific rank as originally defined. In all cases, the tooth formula was much greater than in the typical *pulcherrimus*. The upper jaw is shown in Fig. 5. A holotype specimen has been deposited in the U. S. National Museum Collection, No. 20595.

ACANTHOCEPHALA OF THE GENUS *CORYNOSOMA* FROM THE CALIFORNIA SEA-LION

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In a study of four lots of ACANTHOCEPHALA from the California sea-lion, *Zalophus californianus*, two species of the genus *Corynosoma* Lühe, 1904, have been found. One of these has apparently been previously described from Japanese hosts; the other has been apparently heretofore unrecognized in the literature.

In 1935 Harada described *Corynosoma ambispinigerum* from the small intestine of *Phoca* sp. from North Pacific waters off Japan. In an appendix to his paper Harada stated that *C. ambispinigerum* was a synonym of *C. osmeri* which was described by Fujita in 1921 from immature forms collected from the peritoneal membrane of *Osmerus lanceolatus* taken at Mukawa, Hokkaido, Japan. Harada recognized a discrepancy between Fujita's published description and his own observations on some of the original specimens of *C. osmeri*. According to Fujita the proboscis hooks of *C. osmeri* are arranged "28 in each 19-12 circular" row, whereas Harada by his own observations on some of the same material found 18 longitudinal rows of hooks with 10-11 in each row.

Two of the lots from the California sea-lion contained five specimens of an acanthocephalan which is apparently identical with the specimens that Harada named *C. ambispinigerum*. Accepting Harada's conclusion that *C. ambispinigerum* is not a separate species, the valid name for the specimens under consideration here becomes *Corynosoma osmeri* Fujita, 1921.

The following is a description of *C. osmeri* based upon study of two females

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(apparently neither fully mature) and three males which adds some details omitted by both Harada and Fujita.

Corynosoma osmeri Fujita, 1921
(Figs. 2, 3, 5, 6, 9)

Body approximately 6 mm long divided into two regions: 1) anterior swollen, bulbous region, 2) posterior elongated trunk (Fig. 9). Body spination in two fields: one covering anterior bulbous region and extending but a short distance on ventral surface of trunk; the other around genital pore, which is arranged in about six quincuncial rows in males, and only a few scattered spines in females. Spines on bulbous part of body 28–52 μ long and 5–20 μ in maximum width. Trunk spines 24–44 μ long and 6–8 μ in maximum width. Genital pore subterminal, slightly dorsal.

Bulbous end of body bent ventrally so that proboscis protrudes at obtuse angle from longitudinal axis of body (Fig. 9), as characteristic for most species of *Corynosoma*. Proboscis 651–686 μ long in females, no measurements on males; swollen just posterior to mid-region (Fig. 5); vesicle of unknown origin or function present in swollen area; 176–180 μ wide at anterior end in females, 211 μ in male; 237–246 μ at swollen area in females, no measurements on males; armed with approximately 18 longitudinal rows of hooks, each row with 11 hooks in both sexes. Measurements, in microns, of proboscis hooks as follows:

		Length	Width (at angle)	Length of root
Anterior hooks	♀	44–78	13–16	56–64
	♂	68–72	16–22	44
Conspicuous hooks on swollen area	♀	72–76	28–36	82–86
	♂	64–80	26–28	76
Basal hooks	♀	40–42	6–16	40–60
	♂	no measurements		

Anterior hooks as well as thickened hooks near mid-region of proboscis have strongly recurved roots (Fig. 2). Hooks at base of proboscis have inverted Y-shaped root (Fig. 3).

Proboscis sheath double-walled, no accurate measurements on length and width possible. Lemnisci probably shorter than proboscis sheath.

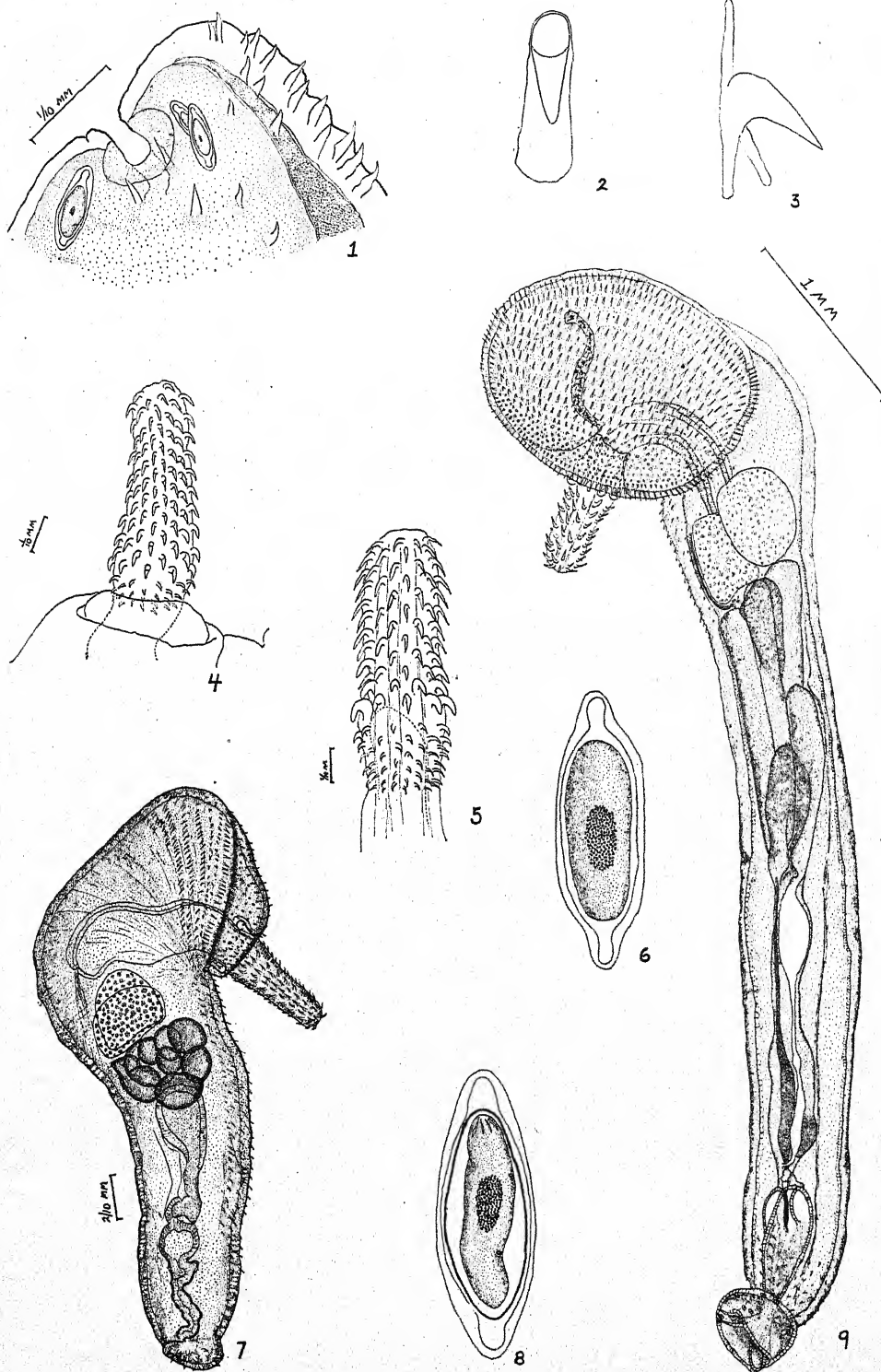
Testes two, situated nearly side by side in anterior half of body near posterior end of proboscis sheath. Cement glands six in number, elongated, clavate in shape immediately following the testes (Fig. 9). Eggs 52–78 μ long and 16–18 μ wide with polar outpocketings (Fig. 6).

In describing the proboscis hooks of *C. ambispinigerum* Harada made no mention of conspicuously thickened or heavy hooks on the swollen area of the proboscis, nor in his figure of the proboscis of this animal did he indicate that any such outstanding hooks were present. Similarly Fujita described no prominent hooks on the proboscis of *C. osmeri*. In the specimens at hand the hooks on the swollen area of the proboscis are conspicuous.

The second species of *Corynosoma* present in the collections from *Zalophus californianus* apparently has been heretofore unrecognized in the literature. For this species the name *Corynosoma obtuscens* is proposed.

Corynosoma obtuscens n. sp.
(Figs. 1, 4, 7, 8)

Specific diagnosis: Body small, almost in form of inverted L, anterior end swollen into bulb and bent ventrally; 2.2–3.0 mm long in females, 2.0–2.8 mm in males. Maximum thickness (dorso-ventral) of posterior body 0.4–0.7 mm in females, 0.3–0.5 in males.



Anterior bulbous part of body on both dorsal and ventral surfaces closely set with spines; trunk with spines only on ventral surface, in females extending all the way to genital pore (Fig. 1), in males most of way but separated from genital spines by area devoid of armature (Fig. 7). Spines on fore part of body 28–52 μ long in females, 32–50 μ in males; spines on posterior trunk of females 28–46 μ long, of males (genital spines excepted) 30–40 μ long. Male genital spines set in about six rows, approximately 30 in number and 29–48 μ long, 12–20 μ in maximum width.

Proboscis bent at obtuse angle from longitudinal axis of body, swollen near base (Figs. 4 and 7), 528–616 μ long in females, about 546 μ in males. Width of proboscis at anterior end 116–156 μ in females, 104–140 μ in males; at swollen region 180–204 μ in females, 144–180 μ in males; at base 160–188 μ in females, 144–160 μ in males.

Proboscis hooks in 16–19 longitudinal rows in females, 17–18 in males, with 12–13 hooks in each row (Fig. 4). Measurements, in microns, of proboscis hooks as follows:

	Length				Width			
	Anterior (from tip to swollen area)		Posterior (below swollen area)		Anterior (from tip to swollen area)		Posterior (below swollen area)	
	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral
♀	46–56	44–56	28–40	16–32	8–18	8–18	4–10	6–12
♂	36–50	40–56	28–30	28	8–16	8–16	6–9	6–8

Proboscis sheath double-walled, no measurements possible. Lemnisci short, flattened and extending about three quarters length of proboscis sheath. Main longitudinal canals of lacunar system lateral.

Testes two, located well forward, side by side, immediately behind proboscis sheath. Cement glands 6 (8?), pyriform, grouped in a cluster closely behind testes. Genital opening subterminal, slightly dorsal in position in both sexes. In female strong sphincter muscle guarding genital opening (Fig. 1). Eggs 68–90 μ in length and 20–28 μ in width with polar outpocketings (Fig. 8).

Host: *Zalophus californianus*, the California sea-lion.

Habitat: Intestine.

Locality: Zoological Gardens, San Diego, Calif.

Types: Holotype male (DL 121.23) and allotype female (DL 121.24) in collection of writer. Paratypes in collection of writer, H. J. Van Cleave in Urbana, Illinois, and deposited in the U. S. Nat. Mus. No. 36843.

Many species of the genus *Corynosoma* have essentially the same proboscis hook formula. If the factors of natural variability and difficulties of accurate observation are taken into account the pattern of the proboscis hooks becomes a poor criterion for the separation of species in this genus, unless the pattern is of such a character as to be distinctive and is beyond the expected variation due naturally or to faulty observation.

C. obtusens differs significantly from five species as to the kind, number and arrangement of the proboscis hooks. In *C. semerme* (Forssell, 1904) there are

FIG. 1. Lateral view of posterior tip of female *Corynosoma obtusens*.

FIG. 2. Frontal view of anterior proboscis hook of *Corynosoma osmeri*.

FIG. 3. Hook from basal portion of proboscis of *C. osmeri*.

FIG. 4. Proboscis of *C. obtusens*.

FIG. 5. Proboscis of *C. osmeri*.

FIG. 6. Egg of *C. osmeri*.

FIG. 7. Male of *C. obtusens*.

FIG. 8. Egg of *C. obtusens*.

FIG. 9. Male of *C. osmeri*.

All figures drawn with the aid of a camera lucida except Figs. 2, 3, 6 and 8.

22-24 longitudinal rows each with 12-13 hooks (Meyer, 1932) and according to Lühe (1911) there are 24-26 longitudinal rows. In *C. reductum* (von Linstow, 1905) there are 24 rows of 10-11 hooks; in *C. hamanni* (von Linstow, 1892) 28 rows of 10 hooks. *C. obtuscens* may be distinguished from *C. turbidum* Van Cleave, 1937 as well as *C. osmeri* Fujita, 1921 by the fact that the latter two species have large conspicuous hooks arming the swollen region of the proboscis.

The remaining species of *Corynosoma* all have hook patterns of approximately eighteen longitudinal rows with nine to fourteen hooks per row. *C. obtuscens* may be distinguished from *C. strumosum* (Rudolphi, 1802), *C. pyriforme* (Bremser, 1824), *C. bullosum* (von Linstow, 1892), *C. tunitae* (Weiss, 1914), *C. osmeri* Fujita, 1921 (syn. *C. ambispinigerum* Harada, 1935), *C. australe* Johnston, 1937, *C. antarcticum* (Rennie, 1907) (syn. *C. sipho* Railliet and Henry, 1907, according to Johnston and Best, 1937), *C. peposacae* (Porta, 1914), *C. phalacrocoracis* Yamaguti, 1939, and *C. mergi* Lundström, 1941 with respect to the kind and distribution of body spines.

C. obtuscens differs from *C. constrictum* Van Cleave, 1918 in that there are no body constrictions such as have been described for the latter.

Yamaguti (1935) records a species of *Corynosoma* which, due to insufficient material, has not been specifically identified. From the fragmentary information given it appears that *C. obtuscens* is distinct from *Corynosoma* sp. of Yamaguti on the basis of hook size and body spination. The hooks of *Corynosoma* sp. are longer and the body spines on the anterior end are in two circular bands.

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THE RATE OF GROWTH OF *ANOPHELES QUADRIMACULATUS* IN RELATION TO TEMPERATURE

HERBERT S. HURLBUT*

Insects in general exhibit an increased rate of growth with a rise in temperature. In particular species the relation of temperature to growth is sufficiently constant to furnish a basis for predicting growth rates at known temperatures. This study was undertaken in order to provide a working basis for predicting the growth rates of *Anopheles quadrimaculatus* under the varying temperature conditions found in nature.

LABORATORY OBSERVATIONS WITH CONSTANT TEMPERATURES

A domesticated strain of *A. quadrimaculatus* was reared at temperatures of $82^{\circ} \pm 1^{\circ}$, $74^{\circ} \pm 2^{\circ}$, and $64^{\circ} \pm 1^{\circ}$ F. Rearing was unsuccessful at $53^{\circ} \pm 1^{\circ}$ F, but eggs and pupae completed their development. Food, consisting of pulverized commercial dog food, was supplied according to the method employed routinely for the stock colony (Crowell, 1940). The observations have been grouped under three headings, the egg stage, the pupal stage, and the complete cycle from egg to adult. In order to facilitate comparison between different temperatures, temperature summation has been employed in the calculation of a time-temperature value, using 50° F as the developmental zero. The data suggest that this approximates the true zero. The average of the time-temperature values at different temperatures has been utilized as a thermal constant. If one assumes that the rate of development is directly proportional to the temperature, the relationship may be expressed by the formula, $a(b-c) = k$, in which a is time, b is temperature, c is the temperature below which development does not occur, and k is the thermal constant. The degree of conformity to this relationship may be judged by the variability of the observed time-temperature values.

Egg Stage

Eggs were collected within one hour after deposition and subjected immediately to the experimental conditions. Counts of larvae were made at hourly intervals throughout the period of hatching. The data are summarized in Table 1.

TABLE 1.—*Egg stage—Anopheles quadrimaculatus*

Time in hours			Temperature degree F.	Total degree-hours for mean period. Develop. zero 50° F	Total eggs hatching	Per cent hatching
Mean	Min.	Max.				
38.1	37	42	82 ± 1	1219	129	82
38.7	37	40	82 ± 1	1238	176	..
54.1	53	60	74 ± 2	1298	116	87
57.9	56	61	74 ± 2	1390	107	..
108.8	104	112	64 ± 1	1509	152	76
357.9	324	420	53 ± 1	1074	135	72
368.1	360	384	53 ± 1	1104	119	83

If one applies the method of thermal summation, assuming a developmental zero of 50° F, the degree-hour value is seen to vary from 1074 to 1509. More closely

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controlled low temperatures might have produced less variation in this figure since a change of 0.5 to 1.0 degree would have given values approximating those found for the higher temperatures. However, if the figures are averaged as they stand, a value is obtained which it is believed is of practical use in estimating the duration of the egg stage at any particular temperature within the range indicated. At extremes of temperature it is well known that the method of thermal summation becomes unreliable. Fig. 1 shows graphically the time-temperature curve obtained

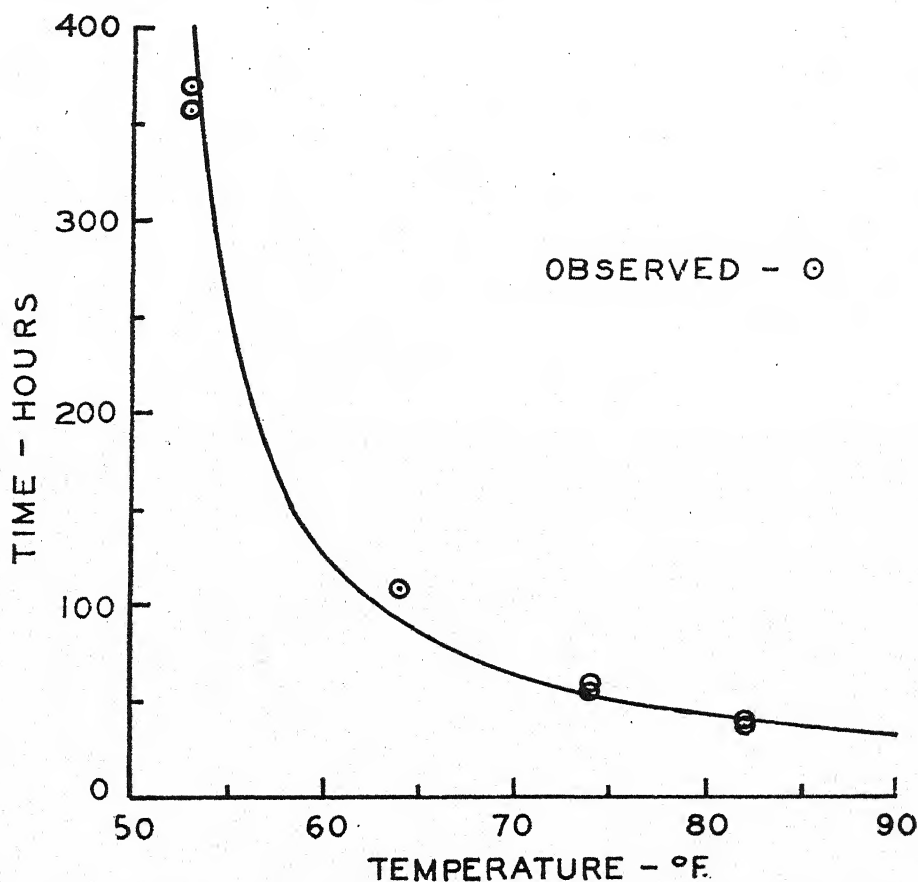


FIG. 1. Time-temperature curve for the egg stage of *Anopheles quadrimaculatus* at constant temperatures, based on the formula: hours (degrees F-50) = 1262. The observed points are indicated.

by using this value, together with the points determined experimentally. The formula employed in constructing the curve is as follows: Hours (degrees F - 50) = 1262.

Pupal Stage

The duration of the pupal stage was observed at $82^{\circ} \pm 1^{\circ}$, $74^{\circ} \pm 2^{\circ}$, and $53^{\circ} \pm 1^{\circ}$ F. The data are presented in Table 2. Again applying the method of thermal summation with an assumed developmental zero of 50° F, the degree-hour value is seen to vary from 930 to 1262. The number of pupae and the number of temperatures are probably too small for accurate determination. However, a trend

TABLE 2.—*Pupal stage—Anopheles quadrimaculatus*

Time in hours			Water temperature degree F	Total degree-hours for mean period. Assumed developmental zero—50° F	Total adults emerging	Per cent emerging
Mean	Min.	Max.				
38.1	37	40	82 ± 1	1219	14	100
52.6	49	55	74 ± 2	1262	11	100
309.9	303	315	53 ± 1	930	11	84

similar to that observed for the incubation period is seen here also. It is of interest to note that the hour-degree value is almost identical to that of the egg stage.

Egg to Adult

The duration of development from egg to adult appeared to be directly proportional to the temperature, at constant temperatures of 82° F and 74° F. The departure at 64° F may have been due to an insufficient number of specimens reared to maturity. The data are presented in Table 3. Although embryonic development

TABLE 3.—*Egg to adult, constant temperature—Anopheles quadrimaculatus*

Time in days egg to adult			Water temperature degree F	Total degree-days for mean period. Dev. zero 50° F	Total adults emerging	Per cent reared
Mean	Min.	Max.				
11.70	11	14	82 ± 1	374	54	42
12.08	11	14	82 ± 1	386	38	22
16.18	15	18	74 ± 2	388	82	70
16.53	15	18	74 ± 2	396	94	88
36.85	28	43	64 ± 1	516	36	24

was completed and hatching took place at 53° F, the larvae failed to develop further, all dying within a few days after hatching, without perceptible growth. The degree-day value secured by averaging the value for the individual groups, excluding the one for 64° F, is 386.

GROWTH RATES AT OUTDOOR TEMPERATURES

Larvae were reared in small cages made of bolting silk (Fig. 2) in a natural breeding place. The cages were 10 inches square by six inches deep, with a top of



FIG. 2. Equipment for outdoor rearing of *Anopheles quadrimaculatus*. One rearing cage is opened showing thermometer.

bolting cloth closed by a zipper fastener. They were fastened to a light wooden frame which fitted into a square opening in a raft made of cypress. Large, burned-out electric bulbs were utilized as floats. The cages were so placed that the water depth in them was about four inches, leaving two inches between the water and the top of the cage. The raft floated freely so the water level inside the cage remained constant. A maximum and minimum self-registering thermometer was suspended horizontally inside one of the cages at about $\frac{1}{4}$ inch below the water surface. Eggs from captive wild females were introduced into a cage about 12 to 18 hours after oviposition. The number of specimens in each stage was counted daily and the maximum and minimum temperatures recorded. The naturally occurring food was supplemented by liberal amounts of the pulverized commercial dog food added daily. An abundant growth of filamentous algae was generally present. In the spring *Spirogyra* and *Ulothrix* comprised most of the growth, but during the summer these were replaced naturally by *Oscillatoria*.

The data from these observations at outdoor temperatures are recorded in Table 4. The first generation was reared from eggs deposited in the spring by overwinter-

TABLE 4.—Egg to adult, outdoor temperatures—*Anopheles quadrimaculatus*

Date, 1941	Time in days egg to adult			Water temperature degree F			Total degree- days for mean period develop. zero 50° F	Total adults emerg- ing	Per cent reared out
	Mean	Min.	Max.	Mean	Min.	Max.			
Mar. 23-									
Apr. 30	33.4 ± 0.24*	30	38	63.9	45	79	465	75	74
Apr. 27-									
May 20†	21.0 ± 0.11	18	23	74.4	63	88	514	80	83
May 19-									
June 4	14.6 ± 0.13	12	16	82.1	73	90	469	46	51
June 7-									
June 27	18.0 ± 0.23	15	20	83.6	77	92	606	36	47
June 26-									
July 13	15.6 ± 0.06	14	17	87.2	80	95	580	119	42
July 29-									
Aug. 16	15.3 ± 0.23	12	18	88.3	84	94	586	52	32
Aug. 30-									
Sept. 18	15.1 ± 0.12	12	19	85.0	80	91	528	128	69
Sept. 14-									
Oct. 4	15.4 ± 0.14	12	20	81.8	76	90	490	194	89

* Standard error.

† Domesticated strain.

ing females. The females had been collected in early November and were kept in a cave throughout the winter. Ten generations were reared in series from March through October. The generation of July 13 to 29 is omitted from the table because the pupae were largely destroyed by predators, and that of October 4 to 27 because of insufficient data. The results, however, were in general agreement with the observations presented. Each generation was derived from captive wild females with the exception of the April 27 to May 20 group which was from the domesticated strain.

A mean temperature for each generational period was calculated by averaging the maximum and minimum daily temperatures. Hourly readings in most instances gave a mean in fairly close agreement with the daily mean derived from the maximum and minimum, though this may well be a source of error in determining the time-temperature value. The product of the mean temperature minus 50 and the average number of days gave a degree-day value which varied from 465 to 606, the

average being 530. A time-temperature curve based on this value is given in Fig. 3, the points being determined by the formula: days (degrees F - 50) = 530. While considerable variation was observed, it would appear that temperature summation based on maximum and minimum daily temperature does provide a working basis for predicting the duration of the life cycle of this species under the varying temperatures occurring in nature. Thus the total degree-days required in early spring

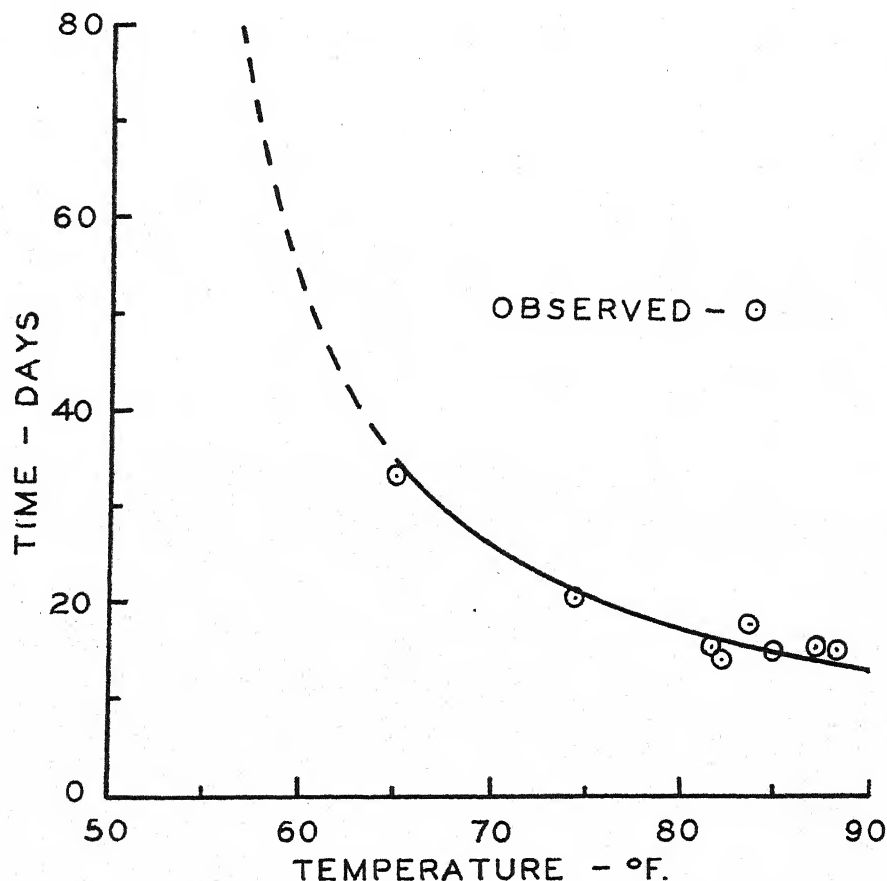


FIG. 3. Time-temperature curve for the complete development of *A. quadrimaculatus* from egg to adult at outdoor temperatures, based on the formula: days (degrees F-50) = 530. The observed points are indicated.

when the range was from 45° to 79° F approximates the value found for mid-summer when there was but slight variation from day to day in the maximum and minimum temperature. Undoubtedly there are important factors other than temperature which are involved, particularly food. An attempt was made to hold this factor as nearly constant as possible. Obviously this could not be done within strictly narrow limits under semi-natural conditions, or even under laboratory conditions, in the present state of our knowledge of the nutritional requirements of this species.

Attention is called to the fact that the time-temperature value at constant temperatures is much smaller than at outdoor temperatures. The method of obtaining

the mean temperature might account for this in part though it is difficult to explain such a wide discrepancy on this basis.

OBSERVATIONS IN THE NATURAL HABITAT

While a detailed study under strictly natural conditions has not been made, a few available observations can be recorded. On several occasions 4th instar larvae were found to be present in a natural breeding place, 6 to 8 days after refilling, subsequent to dewatering of the area. This was during July, August and September when it was observed that the duration of the developmental period did not vary significantly under experimental conditions. In another study (Hinman, 1939) it has been observed that the larval instars of *A. quadrimaculatus* tend to show successive peaks of abundance in its natural habitat. Thus, a peak for 1st instar larvae was followed after 7 to 9 days by one for 4th instar larvae. From the experimental data, the mean duration of larval life during the summer months is calculated to be about 12 days. If one deducts 3 to 4 days for the incomplete 1st and 4th instar periods, the result is in accord with the observations in the natural habitat.

PRACTICAL APPLICATION

The time of initiation of significant *A. quadrimaculatus* breeding in the spring is of vital concern to malaria control workers. In the Tennessee Valley, it is believed that this species passes the winter primarily as inseminated adult females (Hinman and Hurlbut, 1940). They take blood and deposit their eggs in the early spring. As already indicated, the 1st generation reared at outdoor temperatures in this study was derived from overwintering females. Eggs were deposited on March 23, and the generation was completed on April 25. A 2nd generation started on April 27 was completed May 20. This latter date approximated the first appearance of adult *A. quadrimaculatus* in significant numbers in nature. Typically, the population density of this mosquito in the lower Tennessee Valley is characterized by a marked increase sometime during the first three weeks of May. This ordinarily marks the appearance of the species in significant numbers. On the basis of these observations it is suggested that this first marked increase in numbers in the spring is indicative of the appearance of the 2nd generation. This would assume that there is a more or less synchronous development of the 1st and 2nd generations. Conditions in nature appear to favor this, since low temperatures at the beginning of the season tend to delay development of eggs deposited then so that those deposited later, at somewhat higher temperatures, actually are not very far behind. Thus groups started at widely separated dates early in the season would mature at approximately the same time. Observations made on the hatching of eggs at this season tend to support this assumption. It was also observed that larvae hatching before daily average surface water temperatures began to consistently exceed 50° F did not survive well.

The application of the method of temperature summation should thus provide a basis for forecasting the seasonal reappearance of this species in significant numbers. Starting with the date when the average daily surface water temperature in a representative breeding place begins to exceed consistently 50° F, the number of degrees in excess of 50 should be summed daily until the degree-day value of 530 is reached. This would indicate that the season had advanced sufficiently to permit

one generation to reach maturity. Repetition of the process would furnish a guide to the progress of the 2nd generation, against which the initial control measures of the season should probably be directed.

Making due allowance for ovarian development, it is estimated from the data presented that *A. quadrimaculatus* may pass through 9 or 10 generations annually in northern Alabama. From field observations on the adults of this species, Boyd (1930) has estimated 7 or 8 annual generations in North Carolina, the latitude of which is slightly further north.

In the application of control measures the minimum duration of the life stages should be considered. For measures effective against all stages of development the degree-day value of 441 has been calculated from the data provided in Table 4. To determine the approximate minimum time from egg to adult at a known average temperature, the following formula may be used: $\text{days (degrees F - 50)} = 441$. For measures effective against the larval stages only, the degree-day value of 305 has been calculated. To determine the minimum duration of the combined larval stages this value should be substituted in the above formula.

SUMMARY

The rate of development of *Anopheles quadrimaculatus* was observed at constant temperatures in the laboratory and at varying temperatures under outdoor conditions. The observations at outdoor temperatures were made continuously throughout one complete season from March through October. While the data do not support the unqualified assumption that the rate of development is directly proportional to the temperature, it is believed that the relationship is sufficiently close to be of practical value in estimating the duration of the life cycle when the temperature is known. The observations at outdoor temperatures indicate that temperature summation can be utilized as a working basis for prediction of growth rates under natural conditions.

On the basis of the data presented it is estimated that *A. quadrimaculatus* may pass through 9 or 10 generations annually in northern Alabama. The annual reappearance of adults in significant numbers during the first three weeks of May is believed to mark the emergence of the 2nd spring generation. A method is suggested for predicting the time of appearance of this generation when daily maximum and minimum water temperatures are known.

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NATURAL TRANSMISSION OF IMMUNITY AGAINST *TRICHINELLA SPIRALIS* FROM MOTHER RATS TO THEIR OFFSPRING

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It has been previously shown in this laboratory that the specific immunity developed by mother rats against infection with certain species of trypanosomes is transmitted to their offspring, the transfer of protective substance taking place largely or entirely through the immune mother's milk (1-4). In the present paper, the results are presented of a test for the transmission of immunity against a nematode parasite, *Trichinella spiralis*, from mother rats to their young, especially in respect to the route of this transfer. Rats are known to become immune to this parasite as a result of recovery from infection (5-7). The transmission of immunity from mother rats as well as from mother rabbits and hamsters to their young has also been demonstrated (8), although the precise route of such transfer is still obscure.

GENERAL METHODS

The immune mother rats were prepared by mating normal adult females which had been fed 2000 isolated living *Trichinella* larvae. Mothers infected from two to five months prior to parturition seemed able to transfer the most effective level of immunity to nurslings.

Similar quantitative methods were used in the present work as in previous studies upon experimental trichiniasis reported from this laboratory (9). The young rats were infected by mouth with 250 isolated *Trichinella* larvae suspended in nutrient broth to which 20 per cent gelatin had been added. The larvae were administered by syringe through a blunted 22-gauge hypodermic needle lowered into the retropharynx. Some animals were autopsied after five days and their intestines searched for adult worms. This was done by slitting the intestine lengthwise and agitating it in physiological salt solution. The solution was then examined with a low power microscope for the presence of adult worms. The remainder of the rats were killed after 34 days, skinned and eviscerated, and their muscle tissue digested in artificial gastric juice. Samples of the digestate were transferred to Syracuse watch glasses and the number of larvae contained in the samples determined. The number of worms in the entire carcass was then established by calculation.

RECOVERY OF ADULT *TRICHINELLA* FROM THE INTESTINES OF YOUNG RATS NURSING IMMUNE AND NORMAL MOTHERS

Immediately after their delivery, four of nine young of a mother rat immune to *Trichinella spiralis* were exchanged with four of eight freshly delivered young of a normal mother. Seven days later all the young were infected with 250 isolated larvae. After nursing for five days, all the young were killed, and the number of adult *Trichinella* in the intestine determined. The numbers of worms recovered from each animal are shown in Table 1.

Those young which nursed the immune mother yielded fewer adult *Trichinella* than did those which nursed the normal mother. It is particularly significant that this was true no matter whether the young had been born of the immune or of the normal mother. If the young of the normal mother were transferred at birth to the

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TABLE 1.—*Recovery of adult Trichinella spiralis from the intestine of young rats nursing a normal mother or a mother immune to Trichinella*

Young rat no.	Born of mother no.	Nursed by mother no.	No. of adult <i>Trichinella</i> recovered	Percentage recovered as adults	Average percentage recovered
1	900 (Immune)	900 (Immune)	2	0.8	3.0
2			2	0.8	
3			10	4.0	
4			12	4.8	
5			12	4.8	
6	915 (Normal)	900 (Immune)	1	0.4	1.0
7			2	0.8	
8			3	1.2	
9			4	1.6	
10	915 (Normal)	915 (Normal)	55	22.0	24.8
11			59	23.6	
12			67	26.8	
13			67	26.8	
14	900 (Immune)	915 (Normal)	28	15.2	24.4
15			55	22.0	
16			71	28.4	
17			80	32.0	

Young rats infected, when 7 days old, with 250 larvae; autopsied 5 days later.
Young exchanged at birth, before nursing own mother.

immune mother, they became as resistant as were those which had been born of this mother and which continued to nurse her. Those young transferred at birth from the immune to the normal mother were, conversely, as susceptible as were the normal mother's own young which had nursed the normal mother. Therefore, the immunity against *Trichinella spiralis* was, so far as could be determined, acquired by the young entirely after birth, through ingesting the milk of the immune mother.

RECOVERY OF LARVAL TRICHINELLA FROM THE MUSCLES OF YOUNG RATS WHICH HAD NURSED IMMUNE OR NORMAL MOTHERS

Four of eight young born of a rat immune to *Trichinella spiralis* were exchanged at birth with four of eight young born of a normal mother. When 13 days old, all

TABLE 2.—*Recovery of larval Trichinella spiralis from the muscles of young rats which have nursed a normal mother or a mother immune to Trichinella*

Young rat no.	Born of mother no.	Nursed by mother no.	No. larvae recovered from muscles	Average recovery
21	900 (Immune)	900 (Immune)	2,430	10,977
22			11,151	
23			12,996	
24			17,334	
25	916 (Normal)	900 (Immune)	4,788	8,770
26			5,022	
27			6,804	
28			18,468	
29	916 (Normal)	916 (Normal)	45,456	55,944
30			45,942	
31			57,650	
32			74,730	
33	900 (Immune)	916 (Normal)	41,209	51,757
34			52,689	
35			55,188	
36			57,945	

Young rats infected, when 13 days old, with 250 larvae; autopsied 34 days later.
Young exchanged at birth, before nursing own mother.

the young were infected with 250 isolated larvae. After 34 days, they were killed, skinned, and eviscerated, and their muscle digested in artificial gastric juice. The total numbers of larvae in the carcass were then estimated after careful counts upon samples of the digestate. The results are given in Table 2.

The average number of larvae recovered from the muscles of those rats which nursed the immune mother was approximately one-fifth that of the rats which nursed the normal mother. No significant difference in resistance between the young of the two mothers was apparent that could not be explained by the transmission of protective substance through the milk of the immune mother. Regardless of parent, so long as the young nursed the immune mother, they became about equally resistant. Likewise if they nursed the normal mother, they were equally susceptible, regardless of parent.

DISCUSSION

The transmission of immunity against *Trichinella spiralis* from mother rats to their young takes place in the same manner as does that against trypanosomes—that is, largely or entirely by the ingestion of the milk of the immune mother. Little or no transmission occurs while the young are in the rat uterus, by way of the placenta. Although these conclusions seem established, from the data offered, in the case of rat trichiniasis, it is, however, not necessarily true that the same route of transfer operates in the human infection with the same parasite. Most authorities feel, indeed, that in most human infections, acquired immunity is transmitted from mother to young by way of the placenta and not through the milk (10).

SUMMARY

The immunity acquired by rats after infection with *Trichinella spiralis* is transmitted to their young, the transfer occurring largely, if not entirely, through the milk of the immune mother. The young born of normal mother rats promptly become immune if permitted to nurse an immune mother rat. Conversely, the young of an immune mother are as susceptible as the young of a normal mother if they are transferred at birth to a normal mother.

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STUDIES ON THE BIOLOGY OF *ANOPHELES WALKERI*
THEOBALD (DIPTERA: CULICIDAE)*

HAROLD T. PETERS

Anopheles walkeri Theobald is one of four anopheline mosquitoes found in Minnesota. While widely distributed in the state it has been considered rare, but a brief survey in 1939 (Daggy, Muegge and Riley, 1941) and the present studies in 1940 and 1941 have shown it to be very common in the Mississippi River Valley in the southeastern portion of this state.

Experiments of Matheson, Boyd and Stratman-Thomas (1933) demonstrated that *Anopheles walkeri* was an efficient transmitter of *Plasmodium vivax* and Kitchen and Bradley (1936) showed that it was a possible carrier of *Plasmodium falciparum*. In addition to such laboratory evidence, Bang, Quinby and Simpson (1940) reported the capture of a specimen in nature harboring malaria plasmodia. In spite of these findings and the relative abundance of the species in some localities there has been very little work done on the biology of the species.

The studies reported here were made in Wabasha County, Minnesota, in the valley of the Mississippi River. Extensive slough areas and marshes make this locality especially favorable for anopheline breeding. Aquatic plants of both submergent and emergent types abound.

MATERIALS AND METHODS

Eggs of *walkeri* were obtained in the laboratory by confining the adults separately in shell vials (75 × 25 mm) or three or four in wide-mouth pint jars. The tops of the containers were covered with cheesecloth and a small amount of water maintained at the bottom. Adults were fed through the cheesecloth cover without difficulty. In the field, eggs were collected with a dipper and brought to the laboratory in jars. During the winter eggs were collected in the field where places of prolific breeding had been observed and well marked the previous season. Under conditions of this season, ice, snow and parts of emergent stems were taken into the laboratory and hatching awaited.

Larvae were collected with a dipper and transferred with a bent teaspoon to wide-mouth pint jars. Most of the rearing was done in the pint jars although individuals were reared in Syracuse watch glasses. Pulverized Ralston dog biscuit was used for food of the larvae with success.

Adult mosquitoes were collected by three methods. A light trap as designed by the workers in New Jersey (Mulhern, 1934) was run nightly. Adults when biting in the field were taken and excellent results were experienced by collecting those found resting on the sides of a barn and sheds at night where they had been attracted by the domestic animals, especially the pigs. A wide-mouth pint jar, modified for an aspirator to permit of larger catches before a transfer was necessary,

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was used and a flashlight was found indispensable. On one favorable night over 400 specimens were taken in 15 minutes.

DESCRIPTION OF STAGES

Anopheles walkeri lays two kinds of eggs, the summer type which hatches without delay during the summer breeding season and the winter type which does not hatch immediately and is capable of withstanding winter conditions. The presence of these two forms was first noted and recorded by Matheson and Hurlbut (1937) and later fully described by Hurlbut (1938b). These two forms are shown in Fig. 1,

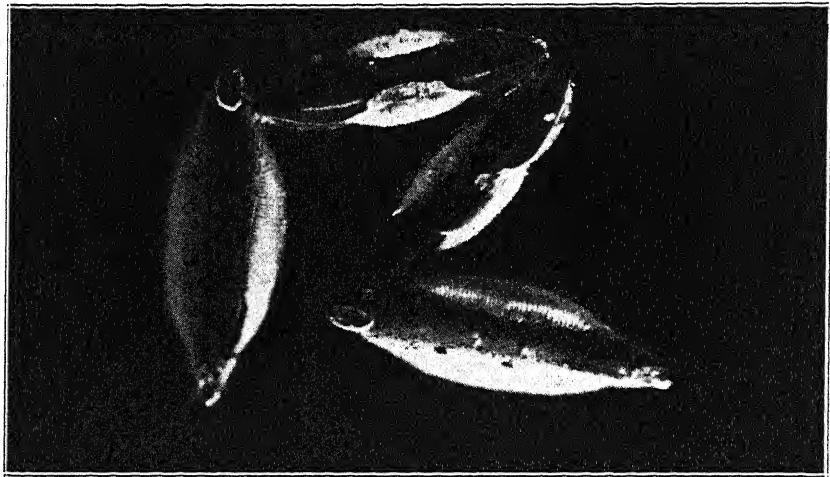


FIG. 1. Summer and winter eggs (the larger) of *A. walkeri*.

the winter eggs being longer, having longer floats and a reticulated exochorion over the dorsal surface. Measurements showed the summer eggs to be $664 \mu \pm \text{S.E. } 0.03$ in length and the winter eggs to be $767 \mu \pm \text{S.E. } 5.4$. These compare favorably with the measurements recorded by Hurlbut. Between the end of the period for the laying of summer eggs and the beginning of winter egg laying, transition forms may be laid which are intermediate between the summer and winter types both in size and structure.

The larvae are very similar to those of *A. quadrimaculatus* Say but may be separated from this species by the small distance between the basal tubercles of the inner anterior clypeal hairs. These hairs are sparsely branched toward the tip. Most of the specimens studied in this region conform to the so-called southern race as described by Bradley (1936). Hurlbut (1938a) has described the larval chaetotaxy of *walkeri* in detail.

The four larval instars are quite easily distinguished even in the field. This may not be done by observing body length alone as this dimension shows a gradual increase through the four instars from 0.9 mm to 7.7 mm. Measurements taken of head capsules of laboratory and field collected specimens show very definite size limitations for the various instars. The scatter diagram (Fig. 2) shows the measurement of the larval head capsules of specimens taken at random from field-collected specimens. The increase in size follows the well known Dyar's Law originally

applied to the head capsules of the LEPIDOPTERA. The average ratio of the measurements of successive instars is 1.6. The size of the head capsule, therefore, is sufficient to determine the stage of growth and application of this principle in the field soon enables the collector to identify readily with the naked eye the larvae to their proper instar.

A characteristic of the pupa which serves to distinguish this species from the

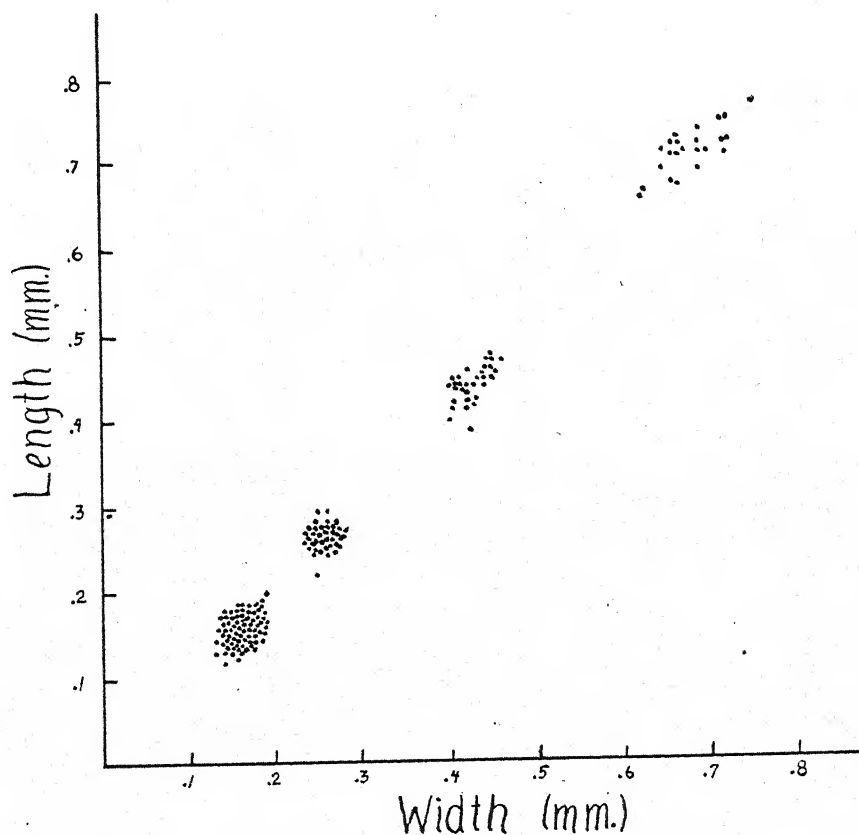


FIG. 2. Measurement of larval head capsules.

other anophelines in this area is the possession of a distinct serration along the outer margin of the paddle. This is shown in Fig. 3.

The adult female was first described by Theobald in 1901 and the male by Matheson and Shannon in 1923. In addition to the usual characters given in keys for *walkeri*, the specimens taken in this region are easily distinguished by their straw colored halteres, the other species possessing black halteres.

LIFE CYCLE

The chief difference in the known life cycle of *Anopheles walkeri* and that of other North American species of the genus is the ability to overwinter in the egg stage. Other phases of the life cycle are similar to those of other anophelines.

Number of generations: In these studies the light trap records for the years 1940

and 1941 were used, supplemented by records of the larval instars taken in the field during the season of 1941. The light trap was operated the entire night each night during the season (April 23 to September 30 in 1940 and May 2 to October 31 in 1941). Larvae were identified to proper instar at each collection in the field.

In 1941 only unhatched winter eggs were taken until April 9 when a few first instar larvae were obtained. Collections made subsequently showed the progress of the growth of these larvae. The adults first appeared in the light trap on May 20, 43 days after the first collection of larvae. A second flight of adults appeared the latter part of June, a third from July 20 through the first week in August and the fourth scattered from August 20 until the end of the season in October. The larval

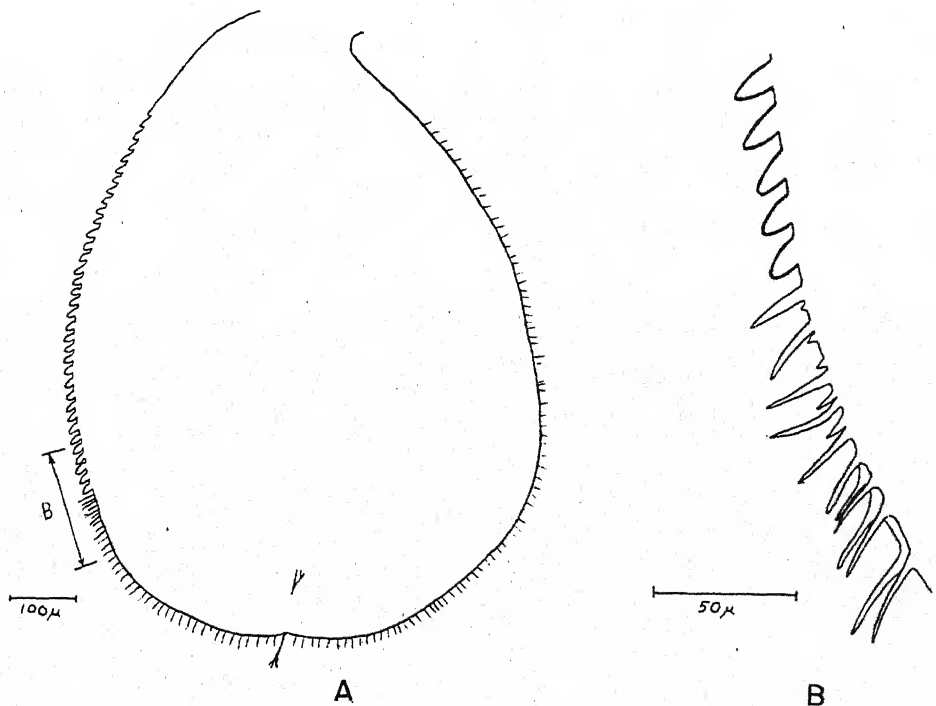


FIG. 3. Pupal paddle of *Anopheles walkeri* Theobald.

collections in each instance showed increased percentage of fourth instars just prior to the flight of adults appearing in the light trap and increase in the first instars immediately after the flights. Thus in the region of southeastern Minnesota there are four generations of *walkeri* per season.

Emergence: A tent of mosquito netting was placed over a square meter of water surface in the breeding area to test the rate of emergence. Over a period of 30 days a total of 115 *walkeri* (32 males and 83 females) were taken in the trap. This represents a per diem emergence rate of 2.8 mosquitoes per square meter of water surface. Collections were made twice daily, at dawn and again just before dusk for one week of the period to test differences in day and night emergence. During that week 21 (5 males and 16 females) emerged during the night and 24 (2 males and 22 females) emerged during the day, indicating that there is little if any preference for day or night emergence.

ECOLOGY

Temperature: Rate of development of *A. walkeri*, like other mosquitoes, varies greatly with the temperature. In the natural habitat there is considerable diurnal variation in temperature. In the laboratory, development from winter egg to adult required 58 days at 15° C, 42 days at 20° C, 31 days at 25° C and 21 days at 30° C. At 35° C the eggs hatched readily but the larvae died before reaching the second instar.



FIG. 4. Favored breeding place of *Anopheles walkeri* in *Leersia* and *Sagittaria*.

Winter eggs were kept in the laboratory at a temperature of 5° C and were kept moist. Experiments subjecting these winter eggs to low temperatures disclosed that they remained viable even when moist and kept at -21° C for a period of 72 hours. The larvae hatched and were perfectly healthy. Experiments at lower temperatures (-25° C and -27.2° C) showed that the larvae were injured. Although they were able to start hatching, they lacked sufficient strength to free themselves from the egg and usually died with the head or head and thorax outside the egg shell.

When the natural habitat of the winter eggs is taken into consideration, it is hardly likely that the temperature would fall low enough to injure the snow covered eggs.

Light: The larvae of *walkeri* are found among emergent vegetation but are more abundant where there is a plentiful supply of light than in densely shaded areas. It

is well known that the adults, although night fliers, very commonly bite in bright sunlight and that they are attracted to lights in large numbers.

Plants: The common emergent plants of this region furnish the habitat for *walkeri*. Among these, especially in the shallow portions of the sloughs, is the cut-grass *Leersia oryzoides*. The occurrence of *walkeri* larvae and *Leersia* together was so frequent that this grass proved to be a valuable indicator in making collections. Fig. 4 shows a typical breeding place in a dense growth of *Leersia* and *Sagittaria*.

Water level: *A. walkeri* normally breeds in water less than a foot in depth. During the 1940 season there was little fluctuation of the river level during the breeding season and there were numerous localities in the sloughs where *walkeri* could be taken at any time during the season. The water level during the last of June and the month of July was essentially the same in 1940 and 1941. At this time some larvae were taken. After the first of August the level dropped so that by the end of the month it had been lowered a foot. After collecting very few larvae on August 2, none of the previously favored places produced larvae. The water gradually receded from the breeding areas and became heavily overgrown with floating plants, principally *Spirodela*, thus precluding anopheline breeding. During this same season the breeding of *walkeri* continued unabated in a second area not influenced by the fluctuating level of the river channel. When a single lowering of the water level during a breeding season has such a deleterious effect, certainly a planned control based on this factor should prove very efficient.

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NUMBER OF LARVAE AND TIME REQUIRED TO PRODUCE ACTIVE IMMUNITY IN RATS AGAINST *TRICHINELLA SPIRALIS**

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Immunity acquired against *Trichinella spiralis* through recovery from a previous infection has been demonstrated in the rat by Ducas (1921), McCoy (1931), Bachman and Oliver-Gonzalez (1936), and Fischthal (1938), in the monkey by McCoy (1932a), in the pig by Bachman and Rodriguez-Molina (1933), in the guinea pig by Roth (1939), and in the mouse by Culbertson (1942). However, the question whether a single small dose of *T. spiralis* will confer immunity and the period of time necessary for its development have never been adequately considered. A preliminary description of this work has appeared in an abstract by Fischthal (1942).

METHODS

Four groups of 8 rats each were fed respectively a single dose of 80, 160, 320, and 640 larvae. Half the animals (4) at each dose level were given test doses of 10,000 larvae at 7 days (A), and the remaining half at 14 days (B). The plan was to examine in turn two of each group 7 days after their test dose for adults in the intestine (X), and the remaining two of each group 42 days after their test dose for larvae in the muscles (Z). In this laboratory the test dose herein used was found to be almost invariably fatal to non-immunized rats.

Controls in regard to the number of adults developing in the intestine were maintained by feeding two rats 80 larvae each, two 160 each, two 320 each, four 640 each, and two 10,000 each. Controls in regard to the number of larval worms in the muscles were not maintained since sufficient control data was accumulated in this laboratory over a period of years by Taylor (1932), Gursch (1933), Edney (1934), and Nolf and Edney (1937).

A total count was made to determine the exact number of larvae in the initial feeding of the rats, and the number of adults recovered from their intestines. Dilution counts (the average number of larvae in 10 one-cc samples multiplied by the total number of cc) were made to determine the test dose of 10,000 larvae, and the number of larvae present in the muscles of the rats.

RESULTS

Results with the experimental rats are shown in Table 1, where A-80-X indicates the rats given an initial dose of 80 larvae tested by 10,000 at 7 days, and examined for adults; B-80-X indicates rats of similar initial dose tested by 10,000 at 14 days, and examined for adults; A-80-Z indicates rats of similar initial dose tested by 10,000 larvae at 7 days, and examined for larvae in the muscles; B-80-Z indicates rats of similar initial dose tested by 10,000 larvae at 14 days, and examined for larvae in their muscles; etc.

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Results with the control rats given an initial dose and killed 7 days later for developing adults in their intestine are shown in Table 2.

TABLE 1

Key no.	No. days after test dose when examined	Adults recovered	Larvae recovered from muscles
A-80-X	3*	5,527	
	4*	5,473	
B-80-X	7†	3	
	7†	1	
A-80-Z	3*	3,185	
	3*	4,244	
B-80-Z	7‡	546	
	42‡		133,550
A-160-X	4*	4,902	
	3*	2,592	
B-160-X	7†	4	
	7†	221	
A-160-Z	42‡		
	3*	5,427	84,450
B-160-Z	20*		81,087
	42‡		77,000
A-320-X	3*	5,749	
	4*	5,293	
B-320-X	7†	2	
	7†	33	
A-320-Z	6*	6,786	
	42‡		86,200
B-320-Z	42‡		103,300
	42‡		113,700
A-640-X	4*	6,300	
	6*	4,425	
B-640-X	7†	5	
	7†	28	
A-640-Z	8*	6,828	
	8*	6,680	
B-640-Z	42‡		153,950
	42‡		149,450

* Died.

† Killed.

‡ Killed accidentally.

DISCUSSION

Only 2 of the 16 rats given either 80, 160, 320 or 640 larvae and a test dose of 10,000 larvae 7 days after the initial infection survived. The 14 that died had a large number of adult worms present in their intestines. Six of these died on the third day after reinfection and the adults recovered varied from 2,592 to 5,749. Four of the 14 rats died on the fourth day after reinfection; the adults recovered varied from 4,902 to 6,300. Two rats died on the sixth day following reinfection and 4,425 and 6,786 adults, respectively, were obtained. The remaining 2 of the

TABLE 2

No. larvae fed	No. adults recovered	% larvae developing
80	24	30
80	34	42
160	22	15
160	32	20
320	125	39
320	172	54
640	52	8
640	26	4
640	491	77
640	534	83
10,000	3,303	33
10,000	3,189	32

14 rats died on the eighth day after reinfection; 6,680 and 6,828 adults, respectively, were obtained from their intestines. These results indicate that immunity had not been established in a period of 7 days, since, as shown in Table 2, the control animals show that approximately 33 per cent of the 10,000 larvae fed were recovered as developing adults 7 days after this feeding. McCoy (1932b) fed 3 rats that were not previously infected 10,800, 9,100 and 11,500 larvae, respectively. Five days later he killed them, recovering from their intestines 5,220, 4,640 and 6,420 adults, respectively. Since in the present series of experiments the majority of rats reinfected with 10,000 larvae died on the third, fourth and sixth days following reinfection, a comparison between the numbers of adults recovered here and the numbers recovered by McCoy shows that the rats reinfected after 7 days did not possess any degree of immunity at all. It appears from the experimental data available that these rats died in the intestinal phase of the disease. Post mortem examination showed intestinal hemorrhage and thrombosis of the mesenteric veins in the majority of cases.

The 2 rats that survived for 42 days the test dose given 7 days after the initial infection had in some way been able to resist the effects of this test dose. The number of larvae recovered from the muscles of the one surviving A-160-Z rat is more than is expected from a dose of 160 larvae, indicating that part of the 10,000 dose produced larvae which entered the muscles. However, the number recovered is approximately one-tenth the amount normally recovered from rats that had survived a single infection of only 9,000 larvae. The number of larvae recovered from the one surviving A-320-Z rat is the amount expected from a rat given a single infection of 320 larvae, indicating that probably no part of the test dose produced muscle infection. The resistance shown by these 2 rats may have been due either to a natural resistance against trichina larvae, to the rapid building up of a partial immunity in a seven-day period which was capable of preventing most of the test dose from producing muscle infection, or to a combination of both.

On the other hand, 15 of the 16 rats given either 80, 160, 320 or 640 larvae and a test dose of 10,000 larvae 14 days after the initial feeding survived. Nine of these rats were killed 7 days after reinfection. The adult worms present in the intestine varied from one to 546 in number. Seven of the 9 rats had less than 33 adults. A definite immunity is herein demonstrated, since, as shown in Table 2, approximately 33 per cent of the 10,000 larvae fed would be recovered as developing adults from non-immunized rats.

The remaining 7 rats given the test dose 14 days after the initial infection showed that immunity had been produced by the previous infection and that the rats were nearly perfectly protected against muscle invasion. It is noteworthy that after immunization by infection the immunity is effective against the intestinal phase of the parasite. The number of larvae recovered from the muscles of the one B-80-Z rat is more than is expected from a single 80 dose, indicating that part of the 10,000 had produced muscle infection; the number recovered, however, is approximately one-seventh of the amount usually obtained from rats that had survived a single infection of only 9,000 larvae. The numbers of larvae recovered from both of the B-160-Z rats are slightly above the expected amount from a single 160 dose; however, they are approximately one-tenth of the number usually recovered from rats that had survived a single dose of only 9,000 larvae. These two animals show that

a higher degree of immunity had been developed with a single dose of 160 larvae than with an 80 dose, although immunity still does not appear to be complete.

The numbers of larvae recovered from the muscles of both B-320-Z and both B-640-Z rats are the expected ones from the feeding of rats with 320 and 640 larvae, respectively, indicating that probably no part of the 10,000 test dose produced muscle infection. Here, complete immunity apparently has been accomplished with only a small number of larvae in a period of 14 days.

SUMMARY

It appears evident from the low numbers of adult *Trichinella spiralis* recovered from the intestines and the low numbers of larvae recovered from the muscles of rats that a single small dose of larvae is capable of producing immunity and that a period of approximately 14 days is adequate to develop this immunity. The feeding of 80 larvae produces a fairly high degree of immunity in 14 days; the 160 dose produces, in the same length of time, a higher degree of immunity; while both the 320 and 640 doses of larvae apparently produce complete immunity in a period of 14 days. The immunity produced is effective against the intestinal phase of the parasite. Little or no immunity is produced within seven days after feeding 80 to 640 larvae.

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MAINTENANCE OF A TREMATODE, *ASPIDOGASTER CONCHICOLA*, OUTSIDE THE BODY OF ITS NATURAL HOST*

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Aspidogaster conchicola is a trematode which usually spends its entire existence within the viscera of fresh-water mussels (Williams, 1942). Faust (1922) has recorded the same parasite from snails and from the digestive tract of a turtle in China. This species, which undergoes direct development with neither alternation of generations nor alternation of hosts, has been considered along with other species of related genera as constituting a distinct subclass of trematodes, intermediate between the MONOGENEA and the DIGENEA. Faust and Tang (1936) have proposed the name ASPIDOGASTREA for this subclass. A fairly full account of the life history stages of *Aspidogaster conchicola* adds support to the argument for recognizing this as a distinct subclass (Williams, 1942). The occasional occurrence of *A. conchicola* in other than the normal host offers evidence that this species is not rigidly fixed in its host relationships. However, the regularity with which it completes an entire cycle in the fresh-water mussels makes it seem plausible that presence in the turtle and in molluscs other than UNIONIDAE is accidental. Appearance in the body of a vertebrate might be explainable through the agency of food chains, wherein infected mussels, eaten by a turtle, permit the worms to become temporarily established in the body of an unusual host. Such secondary attainment of an additional host will be recalled as the basis commonly advanced to explain the origin of alternation of hosts among the parasitic worms.

Besides the forms occurring characteristically in molluscan hosts, there are other species and genera of ASPIDOGASTREA showing normal transitions toward the regular inclusion of a vertebrate host into which the worms must pass before they can reach sexual maturity. Thus in the genus *Cotylaspis*, one North American species, *C. insignis*, lives externally on the body of UNIONIDAE, while another species, *C. cokeri*, is found in the intestine of fresh-water turtles and has been recorded by Simer (1929) from the intestine of a paddle-fish (*Polyodon*). In yet another genus, *Cotylogaster*, an American species attains maturity in the intestine of the fresh-water drum (*Aplodinotus grunniens*). Of the food habits of this fish, Forbes and Richardson (1908: 324) state that it "feeds especially on mollusks, the shells first being crushed by the powerful, paved, millstone-like pharyngeal jaws." This memorandum suggests that in this species the vertebrate host might be superimposed upon a life cycle primitively complete in the molluscan body. This explanation seems particularly plausible since free swimming larval stages are wanting in the ASPIDOGASTREA as a possible means of affecting active transfer from molluscan to vertebrate host.

The versatility expressed by members of the subclass ASPIDOGASTREA in their host relationships suggests the possibility that physiological adjustment between the parasite and its host is not so rigorously fixed as it is among many trematodes. It

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* A contribution from the Zoological Laboratory of the University of Illinois.

therefore seemed probable that members of this group might offer satisfactory material for culture and infection experiments. The availability of *Aspidogaster conchicola*, on which life history observations were being conducted in this laboratory, lead to the selection of this species for further study of host relationships.

Various species of UNIONIDAE, collected from three Illinois streams, were the hosts used in this study. The heaviest infections were found in the mussels from the Sangamon River a few miles northeast of Mahomet, Illinois, and from the Wabash River at West York, Illinois. Lighter infections were encountered in the mussels from the Salt Fork, near Homer Park, Illinois. No attempt has been made to analyze the host relationship in terms of percentages of infection. Kelly has published extensive studies on this topic (1899) with particular reference to the mussel fauna of the Illinois River. In the present series of observations there did not seem to be any species of mussel immune to *Aspidogaster* and the individuals show very wide range in number of parasites without any apparent correlation with size or the locality from which the hosts came. *Amblema costata*, *Pleurobema coccineum*, and *Quadrula pustulosa* were fairly commonly and heavily parasitized in the collections from the Salt Fork and the Sangamon rivers but occasionally uninfected individuals of these species were encountered, while some individuals of *Lampsilis ventricosa* were heavily enough infected to yield numbers of worms sufficient for experimental purposes. Specimens of *Obovaria olivaria* and *Leptodea fragilis* were the chief sources of the worms from the Wabash River. In all of the collections, infections were rarely massive. On a few occasions, 30 to 40 *Aspidogaster* were removed from the pericardial and renal cavities of a single host individual but only rarely were there more than 20 in one mussel.

Two types of experiment were devised, one to test the possibility of maintaining individuals of *Aspidogaster conchicola* outside the body of the host and another type to determine, in preliminary manner, the possibility of individuals of this species surviving artificial introduction into the body of a vertebrate host. Some of the experiments here reported represent single sets of data while others are representative of several trials. None of the experiments has been repeated often enough to provide sound basis for generalizations, but the instances here selected are recorded in the hope that others may find in *Aspidogaster* a suitable material for a detailed program on trematode physiology.

I. THE MAINTENANCE OF *Aspidogaster conchicola* IN VITRO

Several series of experiments were conducted using various media, chief of which were physiological salt solution, Ringer's solution, tissue culture media and mussel blood. Living worms removed from the pericardial and renal cavities of freshly opened mussels, were placed with the medium in 8-dram vials. Most of the vials, after being tightly stoppered, were kept in a refrigerator at temperatures varying from 2 to 9 degrees C, though some were allowed to remain at room temperatures. Vials and instruments were sterilized and reasonable precautions were taken to prevent contamination, but the technique involving repeated washings of the worms was not resorted to, since Gatenby (1937) advises against it in directions for preparing tissue cultures. At reduced temperatures, the trematodes in the various media remained alive for periods varying from about 29 days in a tissue culture medium to a maximum of 75 days in mussel blood. These results, details of which are

presented below, indicate that *Aspidogaster conchicola* may be maintained over relatively long periods of time outside the body of its host and suggest the suitability of this species for physiological studies.

Physiological salt solution.—On October 5, 1940, three adult *Aspidogaster* were placed in a tightly stoppered vial of 0.75% physiological salt solution. After 20 days in the refrigerator all were alive and at room temperatures became active. On November 12, only one of the worms remained alive. After 38 days outside the body of the host, at temperatures between 2 and 9 degrees C, this individual remained inactive when removed to room temperature for examination.

Mussel Ringer's solution.—Six individuals of *A. conchicola* in a stoppered vial of mussel Ringer's solution were alive and apparently in good condition for 21 days (October 7 to 28). At the end of 39 days, at reduced temperature, all but one of the worms were dead. The dead individuals were removed and the one remaining worm lived only one day longer, or a total of 40 days outside the body of its host.

Hedon-Fleig's solution.—Gatenby (1937: 18) gives a formula for this solution, which is recommended for culturing snail tissues. This and another similar solution, which had been found suitable for culturing snail tissues, were tried as media for maintaining *Aspidogaster* in vitro. The results were about the same as for other synthetic media. In different experiments, at low temperatures, the worms lived in these media for from 29 to 38 days and at room temperatures lived for a maximum of 20 days.

Mussel blood.—Preliminary experiments had indicated that *Aspidogaster* may be maintained for 32 days at temperature from 2 to 9 degrees C in a mixture of body fluid and water from the viscera and mantle of freshly opened mussels. This suggested the possibility that pure blood of a mussel might be a satisfactory medium for maintaining these worms in vitro.

To secure a supply of undiluted mussel blood, an emery wheel was used to cut a window in the shell immediately over the pericardial cavity of the mussel (Ellis, Merrick and Ellis, 1931: 510). Heart and pericardial cavity were readily observable through the window. By use of a fine glass pipette, blood was drawn from the pericardial cavity, with a minimum of contamination. Repeated samples of about 1½ cc were taken from the same specimen at intervals of about one week without apparent ill effect upon the donor which was kept in aerated, running water at temperatures ranging from 13 to 21 degrees C.

On November 13, 1940, about 3 cc of mussel blood was placed in a sterilized vial, to which two adult *Aspidogaster* were added. As with the former experiments, the vial was placed in a refrigerator. At intervals of about two weeks, about 3 cc of fresh mussel blood was added, without removal of the blood previously present. On January 28, both worms were still alive. Previous to that date they had become very active at room temperature but on that date they were sluggish and on January 31 both were dead. These two individuals were kept alive in mussel blood for at least 75 days.

Bacterial contamination was fortunately at a low level in the tubes of mussel blood but three species of protozoa, abundant in the cultures, were identified by Dr. R. R. Kudo as *Hexamita inflata*, *Trepomonas agilis* and *T. rotans*.

The foregoing results indicate that mussel blood is far more satisfactory as a medium for prolonged observation of *Aspidogaster conchicola* outside the body of its host than any of the synthetic media that have been tried.

II. EXPERIMENTS WITH *Aspidogaster conchicola* IN VERTEBRATE HOSTS

As mentioned in the introduction to this paper, Faust (1922) has recorded the occurrence, in nature, of *Aspidogaster conchicola* in the digestive tract of a turtle, while some other species of the subclass ASPIDOGASTREA have life histories which normally seem to involve a vertebrate host. These facts, together with the observations on the readiness with which *A. conchicola* seems to adapt itself to existence away from the body of its normal hosts, made it seem desirable to try introducing this species artificially into vertebrate hosts.

On March 3, 1941, four adult worms were injected into the cloaca of each of two small turtles (*Graptemys* sp.). One turtle was killed and autopsied on March 10, but no trace of the worms could be detected in either the cloaca or the intestine. The second individual, autopsied on March 15, likewise gave evidence that the worms had been unable to become established in the cloaca or intestine of the turtles.

By means of a glass tube inserted well down the throat, six mature worms of *Aspidogaster conchicola* were introduced into the stomach of a male turtle (*Pseudemys troosti*), on March 3, 1941. Fourteen days later (March 17) this turtle was killed and upon examination one *Aspidogaster* was found clinging by its ventral sucker to the wall of the turtle's stomach. Frequent examinations of turtles from the same locality had previously established the fact that in nature *Aspidogaster* does not occur normally in turtles of this region. This observation, together with the fact that the turtle was without food from the start of the experiment, makes it seem reasonably certain that the recovered worm was one of those introduced experimentally.

The ability to survive, for at least a time, in the digestive tract of an unusual host, adds further evidence to the flexibility of host relationship expressed by *Aspidogaster conchicola* and makes it seem probable that the instances of occurrence in a turtle reported by Faust and in a paddlefish reported by Simer were the results of accidental introductions when infected mussels were eaten by a vertebrate.

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NOTES ON HIPPOBOSCIDAE. 16. HIPPOBOSCIDAE FROM SOUTHERN BRASIL. WITH THE DESCRIPTION OF A NEW SPECIES OF *LYNCHIA*

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Some time ago the Deutsches Entomologisches Institut sent me nearly a hundred HIPPOBOSCIDAE collected by Mr. Fritz Plaumann in Southern Brasil, at Nova Teutonia, State of Sa. Catharina (27°11' S, 52°23' W). More recently additional Brazilian specimens were received from the same collector and others were presented by Mr. John Lane. These collections are particularly valuable for the careful host records. One of the species appears to be undescribed.

1. *Lipoptena* (*Lipoptenella*) *mazamae* Rondani.—The species is discussed at length in my "Monograph of the Melophaginae" (1942, p. 126). Nova Teutonia, 41 deãlated females off *Mazama tema* Rafinesque and 7 deãlated females off *Mazama americana* (Erxleben) (= *rufa* Illiger) (F. Plaumann Coll.). Unpublished neotropical records: MEXICO: Apatzingan, State of Michoacan, 1,200 ft., off *Odocoileus virginianus* (subsp.?) (R. Traub Coll.). VENEZUELA: San Fernando, off *Odocoileus virginianus gymnotis* (Wiegmann) (Fiasson Coll.).

2. *Ornithoica vicina* (Walker).—Nova Teutonia, two females off *Buteo magnirostris magniplumis* (Bertoni), one female off *Rhamphastos dicolorus* Linnaeus, nine females off *Uroleuca cristatellus* (Temminck) (= *Uroleuca cyanoleuca* Max), one female off *Micrastur ruficollis* (Vieillot), and one female off *Pyroderus scutatus* (Shaw) (F. Plaumann Coll.). Three flies off *Uroleuca cristatellus* and one off *Pyroderus scutatus* carried a mallophagon fixed by the mandibles to the tip of the abdomen. Unpublished neotropical records: ECUADOR: Abitagua, 1,100 m, off a thrush (? *Turdus*), two flies, each bearing a mallophagon (W. Clarke-Macintyre Coll.). CHILE: Valparaiso (Dr. Reed Coll.); Puerto Montt (A. Twoney Coll.). BRASIL: Cara Pintada, State of Parana, off *Micrastur ruficollis* (Vieillot); Humboldt, State of Sa. Catharina, off *Rhamphastos dicolorus* (W. Ehrhardt Coll.). VENEZUELA: Colonia Tovar, off *Scops* sp. (P. Anduze Coll.). GUATEMALA: Uaxactun, Peten, off *Rhamphastos s. sulfuratus* Lesson (J. Van Tyne Coll.). This is the common species thus far called *O. confluenta* in American literature; but Say's species of that name was off a wading bird (See J. Bequaert, 1940, p. 327; 1941, p. 289).

3. *Microlynchia crypturelli* J. Bequaert (1938, p. 346, Figs. 1-3).—Nova Teutonia, one female off *Odontophorus capueira* (Spix), one female off *Crypturellus obsoletus* (Temminck), and three males and six females off *Columba rufina sylvestris* Vieillot (F. Plaumann Coll.). These flies all agree with the type. The occurrence on wild doves is unexpected, but apparently not accidental, as it was taken on at least three of these birds at different dates.

4. *Pseudolynchia canariensis* (Macquart) (Syn.: *maura* Bigot).—Ypiranga, State of S. Paulo, off domestic pigeons (J. Lane Coll.). Unpublished neotropical

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records: MEXICO: D.F. (G. Lasmann Coll.). BRITISH GUIANA: Georgetown, off domestic pigeon (J. Rodway Coll.).

5. *Stilbometopa podopostyla* Speiser.—Nova Teutonia, two females off *Columba rufina sylvestris* Vieillot, one female off *Tinamus solitarius* (Vieillot), one female off *Crypturellus obsoletus* (Temminck), and one female off *Chamaeza brevicauda* (Vieillot) (F. Plaumann Coll.). Ipamiry, State of Goyaz, off turkey (perú), and Goyaz, off dove (J. Lane Coll.). Unpublished neotropical records: MEXICO: San José del Cabo, Lower California, off *Zenaidura macroura* (Linnaeus) (G. Augustson Coll.); La Majada, Munic. Apatzingan, 1,200 ft., State of Michoacan. off a dove (H. Hoogstraal and R. Traub Coll.). VENEZUELA: Las Robles, Guaira, off a white-tailed dove (P. Anduze Coll.). PARAGUAY: Villarrica, off domestic fowl (F. Schade Coll.).

6. *Lynchia fusca* (Macquart).—Nova Teutonia, one female off *Micrastur ruficollis* (Vieillot) and two females off *Buteo magnirostris magniplumis* (Bertoni) (F. Plaumann Coll.). Unpublished neotropical records: PANAMA: Sa. Clara, Chiriqui, off *Bubo virginianus mayensis* Nelson (M. E. McLellan Coll.). BRASIL: Guara-puada, Rio Jordão, State of Paraná, off *Speotyto cunicularia grallaria* (Temminck); Cara Pintada, State of Paraná, off *Otus c. choliba* (Vieillot); Vermelho, State of Paraná, off *Strix hylophila* Temminck; Joinville near Humboldt, State of Sa. Catharina, off "*Bubo crassirostris*" (erroneous name) (W. Ehrhardt Coll.); São Paulo, off *Tyto alba tuidara* J. E. Gary (G. A. Allen Coll.; these specimens were recorded by error in 1933, p. 77, as from San José, California).

7. *Lynchia angustifrons* (van der Wulp).—Nova Teutonia, two females and one male off *Micrastur ruficollis* (Vieillot) and one female off *Ictinia plumbea* (Gmelin) (F. Plaumann Coll.). Unpublished neotropical records: MEXICO: without more definite locality off *Accipiter striatus velox* (Wilson) (G. H. Merriam Coll.); Misantla, off "turkey buzzard" (W. Engelmann Coll.); Motzoronga, State of Vera Cruz, off *Trogon* sp. (L. Bruner Coll.). BRITISH HONDURAS: El Cayo, off *Glaucidium brasilianum ridgwayi* Sharpe, *Ictinia plumbea* (Gmelin) and *Odontotrichis palliatus* (Temminck) (J. Van Tyne Coll.). GUATEMALA: Uaxactun, Peten, off *Accipiter bicolor* (Vieillot) and *Ramphastos s. sulfuratus* Lesson (J. Van Tyne Coll.); Olas de Moca, Dept. Solola (G. P. Engelhardt Coll.). BRITISH GUIANA: Kartabo (W. Beebe Coll.); Issororo, N.W. district, off *Accipiter bicolor* (Vieillot) (G. M. Vevens Coll.). BRASIL: Joinville near Humboldt, State of Sa. Catharina, off *Elanus* sp. and "*Bubo crassirostris*" (erroneous name) (W. Ehrhardt Coll.).

8. *Lynchia plaumanni* n. sp.

Lynchia plaumanni n. sp.
(Fig. 1)

Female.—Head (Fig. 1B) seen in front about one and one-third times as wide as high; frons at its narrowest about one and two-thirds times as wide as eye, measured along inner orbits scarcely longer than its greatest width at vertex, with sides markedly convergent toward lower third; inner orbits (parafrontalia) moderately wide, about one-third of width of mediovertex (frontalia) at its narrowest; frontal bristles few: a lower group of four (three very long) on gena and median row of three to five (one very long) near edge of mediovertex; one very long vertical bristle; postvertex (vertical triangle) much wider than long, the nearly straight or slightly wavy anterior margin longer than the sides, with one or more rudimentary ocelli or depressions taking their place, as described below (Figs. 1D-E); fronto-clypeus broadly and

rather shallowly emarginate medially, the antero-lateral angles moderately produced. Palpi moderately long, nearly half height of head.

Thorax (Fig. 1C) of usual shape; anterior margin nearly straight in the middle; humeral lobes slightly longer than wide, moderately prominent, bluntly rounded at apex, with one very long bristle and many shorter setae. Mesonotum on each side with two transverse patches of short setae, behind humeral lobe and before scutellum; a longer seta in prescutellar patch; a very long prealar and a similar postalar bristle on each side; dorsal portion of mesopleura (notopleura) with scattered short setae and one very long bristle near posterior corner. Scutellum semi-elliptical, hind margin evenly convex, fringed at edge with soft hairs and before this with a row of fine short bristles; one long scutellar bristle in each corner; metepimeron with a few, short setae. Legs without distinctive features; fourth tarsal segment of fore legs normal. Wing (Fig. 1A) rather short and broad; microtrichia covering the membrane except for small basal area in anal cell (Cu + 1st An) and most of axillary cell (2d An); costa moderately swollen beyond tip of first longitudinal vein; first basal cell (R) very long, narrow, parallel-sided; second basal cell (M) slightly less than half length of first, closed by nearly vertical anterior basal cross-vein (M_3); subcosta (Sc) incomplete, not reaching costa; costa and basicosta densely setulose; all other veins bare.

Abdomen with usual broad, short, transversely losange-shaped basal, sclerotized tergite, bearing numerous short, stiff setae on sides and soft hairs in middle; preanal (dorsal) sclerite transverse, large, reniform, bearing few short setae and in each apical corner 7 or 8 very long, stiff bristles; remainder of dorsum forming one sclerotized area, without differentiated tergites, dull, microscopically cross-striate, almost without pilosity; sides and venter soft, uniformly and fairly densely covered with short setae; ventrally, two broad sclerites more or less defined on each side over basal half, hind margin of second evenly and broadly rounded off.

Male.—Extremely similar to female, even in width of frons and structure of abdomen; preanal sclerite has same shape; but basal sclerotized tergite is produced at sides into triangular lobes.

Length (of both sexes) from tip of fronto-clypeus to apex of scutellum, 2.7 mm; of wing, 5.5 mm; width of wing, 2 mm (total length of dry specimen, slightly over 4 mm).

Locality.—BRASIL: Nova Teutonia, female holotype and male allotype off *Odontophorus capueira* (Spix); paratypes of both sexes off same host (2 ♀, 1 ♂), off *Crypturellus obsoletus* (Temminck) (1 ♀), and off *Chamaeza brevicauda* (Vieillot) (1 ♀); all collected by F. Plaumann. BRITISH GUIANA: River Paruni, female paratype off *Crax nigra* Linnaeus (= *alektor* Linnaeus). BOLIVIA: St. Elena, Huachi, Rio Beni, male paratype (W. M. Mann Coll.).

Type specimens.—Holotype and allotype at Mus. Comp. Zoöl., Cambridge, Mass.; paratypes at Deutsches Entom. Institut, Berlin-Dahlem, at British Museum, and at U. S. Nat. Mus.

An unusual feature of *L. plaumanni* is the presence of either rudimentary ocelli or ocellar pits, which could be traced in all 9 specimens (Figs. 1B and D-E). Their development varies. There may be only three pits in which no true ocelli can be seen, the pits being often more or less connected or sometimes forming one horse-shoe-shaped depression. The anterior ocellus can rarely be detected, but the two posterior ocelli are often outlined, though small and flat. It is extremely doubtful that any of them are at all functional. As *L. plaumanni* is in every other respect a true *Lynchia*, I can attach no particular taxonomic importance to these rudimentary ocelli. Nevertheless, *L. plaumanni* bridges the gap between *Lynchia* and the genus *Ornithophila* Rondani, at any rate as represented by *Ornithophila maquilingensis* Ferris (1924, p. 392, Figs. 1-2), of the Indo-Australian Region. Of that species I have studied specimens from the Philippines, Indo-China, Ceylon and Queensland, and it has also been reported from Java. It has the ocelli completely formed, though small, the anterior ocellus in particular being minute. Moreover, *L. plaumanni* is certainly distinct specifically, as it differs also in the extent of the bare area of the wing membrane. In *O. maquilingensis*, the wing is uniformly covered with microtrichia, except for a very narrow marginal area in the axillary cell. The paratype of *L. plaumanni*, from Bolivia, was mentioned by Aldrich (1923, p. 78) as running out in his key to *Ornithophila*.

In *Microlynchia*, as well as in some species of *Ornithomyia* (*O. fur* Schiner = *O. inocephala* Ferris), the ocelli are also in the process of disappearing. *L. plaumanni* and *O. maquilensis* have merely retained one of the primitive characters of the ancestral stock of the genus *Lynchia*. Unless the unrecognized genotype, *Ornithophila vagans* Rondani, presents some other unusual feature, I should regard *Ornithophila* as no more than a subgenus of *Lynchia*. Unfortunately *Ornithophila* is the older of the two names and will have to replace *Lynchia*.

In the revised key to the New World genera of HIPPOBOSCIDAE, which I published in 1940 (pp. 312-313), the second alternative of couplet 12 should be amended to read "Ocelli absent or vestigial"; instead of "Ocelli absent."

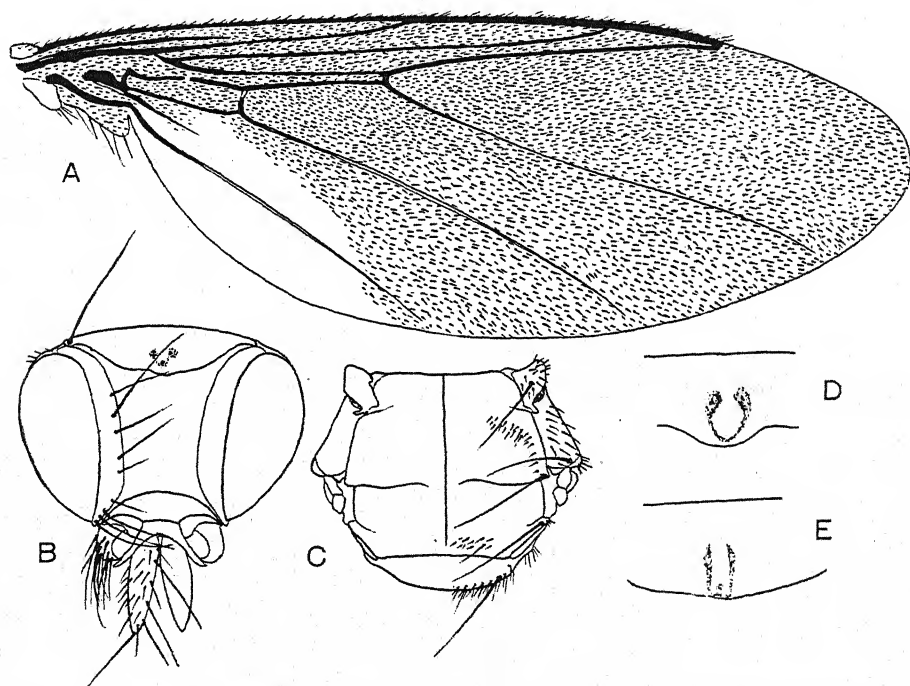


FIG. 1. *Lynchia plaumanni* J. Bequaert. A-C, female holotype: A, wing; B, head in front view; C, thorax from above. D-E, ocellar area of two paratypes.

The following key compares the described and recognizable small American species of *Lynchia* (wing 6.5 mm or less in length).

1. Frons very narrow in both sexes, at most as wide as an eye, usually narrower; inner orbits with few frontal bristles, placed in one row. Entire axillary cell and posterior half of anal cell bare. Palpi long, about one-half of the height of the head. *L. angustifrons* (v. d. Wulp). 2
2. Frons always much wider than an eye in both sexes. 2
2. Wing membrane covered with microtrichia except for a very narrow posterior marginal area of the axillary cell. Inner orbits with many frontal bristles in more than one row. Head subcircular. Palpi very short. Frons nearly three times as wide as an eye. *L. holoptera* (Lutz, Neiva and Costa Lima). 3
- At least posterior half of axillary cell devoid of microtrichia. Head distinctly wider than high. 3

3. Anterior sclerite of mesonotum (prescutum, before transverse suture) with three strong bristles on each side, in the posthumeral patch of setae. Inner orbits with many frontal bristles in more than one row. Only entire axillary cell devoid of microtrichia. *L. hirsuta* Ferris.

No strong bristles in the posthumeral patch of setae on prescutum. 4

4. Only axillary cell either entirely or mostly devoid of microtrichia. Inner orbits with many frontal bristles in more than one row. Palpi short, much less than half the height of the head. No rudimentary ocelli.

. *L. albipennis* (Say).

Most of axillary cell and part of basal area of anal cell devoid of microtrichia.

Inner orbits with few frontal bristles in one row. Palpi long, nearly one-half the height of the head. Rudimentary ocelli present. . . . *L. plaumanni*.

I regard *Olfersia botaurinorum* Swenk (1916), *Olfersia scutellaris* Swenk (1916) and *Olfersia palustris* Lutz, Neiva and Costa Lima (1915) as synonyms of *Lynchia albipennis*.

Olfersia propinqua Walker (1849), from Jamaica, was probably based on a small species of *Lynchia* (length of body $1\frac{1}{2}$ English line = 3.17 mm) or perhaps on a *Microlynchia*. The description mentions no character now used to separate species in HIPPOBOSCIDAE.

9. *Ornithoetona erythrocephala* (Leach).—Nova Teutonia, one female off *Ictinia plumbea* (Gmelin) (F. Plaumann Coll.). São Paulo (J. Lane Coll.). Unpublished neotropical records: MEXICO: without more definite locality. PANAMA: El Volcan, Chiriqui, off a pigeon (C. B. Worth Coll.). VENEZUELA: Caracas (G. Umer Coll.); Merida, off a hawk and off *Accipiter chionogaster venezuelensis* Swann (P. Anduze Coll.); Nanguata Peak, off *Columba albilinea* Bonaparte (E. G. Holt Coll.); Zumbador, Estado Merida, off a pigeon (J. D. Smith Coll.); Rio Charaito, Estado Merida (G. Vivas-Berthier Coll.). BRASIL: Rio Grande do Sul (Stiegmayer Coll.). BOLIVIA: Incachaca, 2,500 m, off *Margarornis squamigera* (Lafresnaye and d'Orbigny) (J. Steinbach Coll.). PERU: Yurimaguas, E. Peru, off *Claravis godefrida* Temminck (= *Peristera geoffroyi* Temminck). ECUADOR: Baños, Oriente, off a small owl (W. Clarke-Macintyre Coll.).

10. *Pseudornithomyia ambigua* Lutz, Neiva and Costa Lima.—São Paulo, off a swallow (J. Lane Coll.). Unpublished neotropical records: VENEZUELA: El Valle, D.F., off *Iridoprocne albiventer* (Boddaert) (C. H. Ballou Coll.); Macuto, Caracas, off *Pygochelidon cyanoleuca* (Vieillot) (N. Dearborn Coll.).

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DEVELOPMENT OF EYE FLUKES OF FISHES IN THE LENSES OF FROGS, TURTLES, BIRDS, AND MAMMALS¹

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About a century after von Nordmann (1832) first found strigeid metacercariae occurring in the eyes of fishes, La Rue, Butler, and Berkhout (1926) demonstrated that they were common habitats for species of these larval trematodes. Strigeid metacercariae have also been reported from the eyes of certain amphibians (Kelley, 1934). Various parts of the eye may be parasitized, depending upon the species of worm. This paper deals with metacercariae of the genus *Diplostomulum* that live only in the cortex of the lens, and is a report of experiments in which it has been found that worms ordinarily developing in the eyes of fishes, and in many cases causing complete blindness, will grow in the lenses of both cold- and warm-blooded animals representing four other classes of vertebrates.

The experiments were performed as an outgrowth of studies on the life history and control of eye flukes in the New Jersey State Fish Hatchery at Hackettstown. The trematodes represented at the Hatchery appear to resemble both *Diplostomum flexicaudum* and *D. indistinctum* (Ferguson and Hayford, 1941), and studies now in progress may show that these recognized forms are variants of a single species. All cercariae used in exposure experiments resembled closely, as far as morphology and behavior were concerned, the cercariae of *D. flexicaudum* (Cort and Brooks, 1928).

MATERIAL AND METHODS

The forked-tail strigeid cercariae were obtained from two sources; a small group of naturally infected snails (*Lymnaea stagnalis*) collected near Minneapolis, Minnesota, and laboratory-reared snails (*Lymnaea palustris*) experimentally exposed to miracidia of the Hackettstown trematodes. The snails were placed in milk bottles containing small amounts of aquarium water and cercariae which emerged were concentrated by centrifuging for a few minutes at low speed. The centrifuge tubes employed were made in this laboratory and are similar to a Hopkins vaccine tube (A. H. Thomas No. 3004) in that they are provided at the bottom with a short slender extension. The tubes have a strong constriction just above the extension and a greater capacity than the Hopkins vaccine tube. After centrifuging, the water in the large part of the tube can be poured out quickly without loss of the worms which collect in the extension.

The cercariae are plankton organisms which remain suspended in the water and the only swimming motion exhibited is a periodic and energetic movement upward. From the higher position they slowly settle after becoming quiescent. Natural fish hosts of *D. flexicaudum*, e.g., suckers, trout, minnows, etc., become infected as they swim into direct contact with the larval worms.

Bluegill sunfish acquire few eye flukes in nature and only a small number of

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cercariae reach the eye when these fish are exposed over several hours to thousands of cercariae in a small container. However, heavy infections can be obtained in the bluegill in at least three ways. (1) If hundreds of cercariae in a small amount of water are injected intraperitoneally into a fish, a varying number of worms usually reach the lens of each eye, where they develop normally and as rapidly as in the eyes of the more susceptible hosts. (2) Even greater numbers of cercariae will arrive in the lenses if a few drops of the suspension are injected into the orbit through the membrane surrounding the eyes. (3) When several drops of the concentrated suspension of cercariae are dropped down the open end of a pipette held tightly over the cornea for several minutes, many worms are also able to reach the lens. With the last method the pipette must be large enough to allow a considerable portion of the outer surface of the eye to be exposed to the cercariae. The pipette is placed in position and the cercariae are added to it while the fish is held out of water. The fish may then be held under water for 10–15 minutes with the pipette in place, by which time many cercariae will have had time to enter the eye.

This method of direct exposure of the eye to the larval worms was useful not only with the bluegill but with most of the animals mentioned in this paper. The diameter of the pipette employed was adapted to the size of the eye. Mice and rats were kept under anesthetic (ether) during the time of exposure, which for all the animals varied from 8 to 15 minutes. In the experiments only one eye was exposed.

Frog tadpoles used in the experiments were allowed to swim in water with the cercariae. Here, of course, both eyes could become infected. Other tadpoles which were examined as controls had no strigeids in their lenses.

EXPERIMENTS AND OBSERVATIONS

Employing the methods described above the following species of animals were exposed to the two strains of cercariae: frog tadpoles—*Rana pipiens* (probably); frogs—*Rana pipiens* (adults); turtles—small green, species (?), and painted—*Chrysemys picta* (small); chicks (2–4 days old); ducklings (3 days old); laboratory white mice (6 weeks old, and adults); white rats (young adults); guinea pigs (adults); rabbits (young adults); a sheep (1 year old); and a pig (2–3 months old). While all the animals did not develop metacercariae, representatives of each species, except the sheep and pig, became positive for eye flukes. Tables 1 and 2 summarize the data and show the interval of time between exposure to cercariae and examination of the lenses, the appearance of the lens at the time of removal from the eye, the number and condition of the worms found, and the size of some of the live metacercariae. Animals included in Table 1 were exposed to cercariae from the naturally infected Minnesota snails, while those in Table 2 were exposed to the Hackettstown material. Table 3 is a summary of all experiments and shows the numbers of animals which were positive or negative for metacercariae.

An experiment was attempted in which hundreds of cercariae were injected intraperitoneally into a guinea pig and four mice. After one day no worms had reached the eyes of any animal. This may mean that the cercariae are unable to survive and migrate in the bodies of these warm-blooded vertebrates. In another experiment several hundred cercariae were placed inside the lower eyelid of a rabbit. The procedure was repeated with two mice. Examination of the lenses two days later revealed no parasites. The cercariae must have been prevented from

TABLE 1.—*Results of experiments in which cercariae of Diplostomum flexicaudum from naturally infected Lymnaea stagnalis were applied to eyes*

Exper. No.	Species of animal exposed to cercariae	Interval between exposure of eye to cercariae and examination of lens	Condition of lens at time of examination	No. of metacercariae found		Range in body size of some live metacercariae after fixation†	
				Alive and active	Dead	Length	Width
		<i>days</i>				<i>mm.</i>	<i>mm.</i>
9E	Tadpole	23	Opaque and flaccid (one lens)	1	4		
5B	Frog	19	Normal	0	6		
5C	"	57	"	10	0	0.25-0.35	0.13-0.17
7F	Turtle (green)	35	"	1	0	0.32	0.13
11	Turtle (painted)	15	"	4	0		
3M	Chick	37	"	2	4	0.23-0.25	0.08-0.09
7E	"	45	"	0	3		
8C	Duckling	16	"	1	0	0.29	0.13
3A	Mouse	26	"	5	0	0.45-0.50	0.16-0.20
3B	"	33	Opaque and very flaccid	11	0	0.45	0.17
3E	"	48	" " " "	0	2		
3 G.1	"	48	" " " "	4	1		
3 G.2	"	97	Normal	1	0		
3H	Rat	27	Opaque and very flaccid	16	0	0.20-0.40	0.10-0.17
3I	"	34	" " " "	11	0		
7A	"	48	" but firm	10	0		
7B	"	55	Normal	1	0		
3J	Guinea pig	33	Opaque and very flaccid	40*	10		
10A	"	42	Normal	0	5		
3K	"	63	Opaque and very flaccid	0	15		
3L	Rabbit	34	Normal	1	10		

* These metacercariae injected into duodenum of a 2-day-old chick. One normal adult worm with eggs recovered from intestine after 7 days. Metacercariae from experiments 3B, 3 G.1, 3I, and 7A were also injected into chicks, but no adult worms recovered.

† 10 measurements made where possible.

entering the eye either because of mechanical obstacles or because they were killed by the eye secretions.

In two instances an adult worm was obtained when live metacercariae from the lenses of a frog and a guinea pig respectively were injected directly into the duodenum of chicks. This indicates normal metacercarial development in these "abnormal" eyes, and is of particular interest because the chick is not the natural host of the adult trematode and very few adult strigeids are obtained even when flukes from the eyes of fishes are fed. Since the live metacercariae recovered from the eyes of various hosts in these experiments were normal in appearance and behavior, it may be assumed that numbers of them were physiologically mature when

TABLE 2.—*Results of experiments in which strigeid cercariae from experimentally infected Lymnaea palustris were applied to eyes*

Exper. No.	Species of animal exposed to cercariae	Interval between exposure of eye to cercariae and examination of lens	Condition of lens at time of examination	No. of metacercariae found		Range in body size of some live metacercariae after fixation†	
				Alive and active	Dead	Length	Width
		<i>days</i>				<i>mm.</i>	<i>mm.</i>
6A	Tadpole	6	Normal (2 lenses)	2	0		
6B	"	18	" (2 ")	3	0		
2A	Frog	19	Opaque but firm	175	0	0.18-0.22	0.07-0.12
2B	"	50	" and very flaccid	40*	15	0.26-0.33	0.08-0.10
1D	Mouse	55	" but firm	4	0	0.30-0.37	0.10-0.17
3A	Rat	50	Normal	1	1	0.30	0.12
3B	"	57	"	5	0		

* 30 of these metacercariae injected into duodenum of a 2-day-old chick. One normal adult worm with eggs recovered after 7 days.

† 10 measurements made where possible.

TABLE 3.—Summary of all experiments showing animals which were positive or negative for metacercariae

Species of animal exposed to cercariae	Cercariae from <i>Lymnaea stagnalis</i>		Cercariae from <i>Lymnaea palustris</i>	
	Positive	Negative*	Positive	Negative*
Tadpole	1	3	2	3
Frog	2	0	2	0
Turtle (green)	1	0	0	1
Turtle (painted)	1	0	0	0
Chick	2	0	0	2
Duckling	1	1	0	0
Mouse	5	6	1	2
Rat	4	0	2	0
Guinea pig	3	3	0	0
Rabbit	1	2	0	2
Sheep	0	1	0	0
Pig	0	1	0	0

*In all cases the lenses were normal in appearance.

removed from the lenses several weeks after exposure of the eyes to cercariae.

The mortality which occurred among the metacercariae in some of the "abnormal" lenses after they had reached morphological maturity might have been greater if the worms had been allowed to remain in the lenses for longer periods, such as several months. Dead eye flukes were usually disintegrating when recovered from the lenses. Strigeid metacercariae do live for many months, perhaps years, in the eyes of fishes, particularly when they are few in number and when the lens is not severely damaged. However, dead and disintegrating metacercariae may be found in fish eyes when the lens has become flaccid and opaque. There is an indication (Table 1) that the eye worms survive longer in mice and rats than in guinea pigs.

Sizes of metacercariae recovered alive and fixed in hot alcohol-formalin-acetic solution were studied (Tables 1 and 2). There is a considerable variation in eye flukes from a single lens just as between metacercariae from the eyes of different species of animals. The range in size of the worms in Table 1 is slightly greater than of those in Table 2. This may have been due to the fact that cercariae released by *L. stagnalis* were larger than those obtained from infected *L. palustris*. Then too, there is still some question as to the specific identity of the cercariae from the two snail hosts which represent strigeid material from Minnesota and New Jersey. The evidence in Tables 1 and 2 suggests it would be premature to state that any marked size variations exist between the worms from the eyes of cold- and warm-blooded animals when they were exposed to cercariae from the same source. This is further emphasized by the fact that metacercariae from the eyes of rainbow trout, bluegill sunfish, and blackhead minnows exposed to cercariae from either *L. stagnalis* or *L. palustris* varied considerably among specimens in each collection. There was not, however, a marked variation in the size range of worms in the different collections from the various species of fish hosts. These sizes correspond rather closely with many of those given in Tables 1 and 2.

Undoubtedly, the environment in which the metacercariae develop and the source of the cercariae are important factors in determining the size attained by the mature eye flukes.

Pathological Effects of the Eye Flukes

The lenses were often seriously affected by the presence of the eye flukes unless the number present was small (Tables 1 and 2). In some cases where only a few metacercariae were present much harm was done. The initial damage to the lenses

brought about an opaqueness of the cortex and nucleus. In those lenses which became flaccid the cortical substance was partially liquefied. The lens capsule then served to retain this material and the more solid nucleus of the lens.

In some of the rabbits, guinea pigs, rats, and mice, the corneas became opaque within a day or so after exposure to the cercariae. This condition usually persisted. Some of these eyes contained damaged lenses, but in others the lenses were normal in appearance. Corneal damage may have occurred at the time of exposure due to irritation caused by the pipette and cercariae, or secondary organisms carried in by the worms as they penetrated the eye.

The affected eyes resembled closely those found in fishes parasitized by numbers of metacercariae as reported by Ferguson and Hayford (1941). However, diseased lenses usually became evident much earlier in the warm-blooded animals than in fish, and in these warm-blooded forms severe damage to the lens resulted from the presence of only a few worms, whereas it usually required large numbers of metacercariae to cause blindness in fishes.

DISCUSSION

Information at hand appears to show that so far as fishes are concerned the lens of the eye is the only tissue in which the cercariae of *D. flexicaudum* can survive and develop. The larval trematodes will penetrate eyes of fishes from which the lenses have been removed, but do not survive more than about a day.

The fact that these metacercariae will develop in animals representing so many classes of vertebrates suggests that the lens materials in the various eyes are, to a certain extent, rather similar in nature. As a matter of fact, tests show that the lens proteins of more or less closely related vertebrates within a class are apparently similar (Markin and Kyes, 1939), but differences have been demonstrated in lens materials of animals belonging to separate classes (Ecker and Pillemer, 1940). These chemical differences are evidently not great enough to make the lens substance of any of the particular animals mentioned here unsuitable nourishment for the developing metacercariae.

Obviously the observations presented here indicate that the metacercaria of *D. flexicaudum* is not strictly host-specific. This is further emphasized in the papers of Cort and Brackett (1937) and Olivier (1940a), who reported that metacercariae of this form sometimes develop precociously in old sporocysts of lymnaeid snails and that these will develop into normal adult trematodes in the chicken's intestine. In certain snails, therefore, some physiological condition permits the larval worms to develop beyond the cercarial stage, and one is tempted to speculate that the environment in older sporocysts in the invertebrate host is similar to that in the cortex of the vertebrate lens.

It is not unique among strigeid trematodes that the metacercariae of *D. flexicaudum* can live and grow in the eyes of such a variety of hosts. Szidat (1929) reported metacercariae of *Cotylurus cornutus* from the hermaphroditic glands of snails and reproductive organs of leeches. A considerably wider range of intermediate hosts for a strigeid metacercaria is listed by Wallace (1939). In China, the tadpoles and adults of several species of frogs, snakes of several species, a skink, and a shrew were found to be naturally infected with larval *Pharyngostomum cordatum*. Metacercariae of this species recovered from frogs and snakes were fed

to rats, chicks, ducklings, and an owl. Those which survived did not develop into adults, but migrated into the muscles and again became encapsulated. The frog is perhaps the only required intermediate host, and snakes may acquire their infection by eating frogs which harbor encapsulated metacercariae already fully developed. Whether cercariae develop only in frog tissues remains to be seen, but it is evident that metacercariae can survive in the gut of many hosts, migrate out into the muscles, and there find locations suitable for survival even though some of the hosts are warm-blooded.

Representatives of various invertebrate and vertebrate classes have also been reported as hosts for the metacercariae of *Strigea vaginata*, *Apatemon gracilis*, and *Prohemistomum vivax* (Walton, 1938; Olivier, 1940b).

Since the eye flukes of fishes develop in the lenses of mammals, and frequently cause blindness, the question arises whether these worms are able to establish themselves and do damage in the eyes of man. There is probably ample opportunity for large numbers of bathers to be exposed to the cercariae of *D. flexicaudum* in the lakes of Michigan, Wisconsin, Minnesota, and surrounding regions both in the United States and Canada (Cort, McMullen and Brackett, 1937). Dr. A. C. Woods, Ophthalmologist-in-Chief, Johns Hopkins Hospital, in a personal communication, informed the writer that there appears to be no published record in this country of larval trematodes having been found in human eyes. Ward (1918) cites no cases for North America, but both he and Salzer (1907) mention a few instances in which trematodes have been found in the eyes of Europeans. None of the worms mentioned appears to be a strigeid. It is possible that the ocular secretions and thickness of the tissues of the eye in larger animals may be effective barriers to the occasional cercariae which might otherwise be able to parasitize people while bathing near the shore in freshwater lakes. It should be recalled also that there was no survival and migration to the eye when cercariae were injected into mice and one guinea pig, nor was there penetration of the eye by large numbers of worms placed under the lower eyelid of mice and a rabbit. That the cercariae of *D. flexicaudum* may be occasionally dangerous to man seems therefore to be rather remote.

If, however, eye flukes ever become established in the eyes of bathers their presence might not be detected for some time. Furthermore, aside from irritation that probably would result from the penetration of the cercariae through the tissues, no apparent damage to the lens might be caused by developing metacercariae (Table 1, experiments 3A, 10A, 3L). On the other hand, a severe disturbance in the lens might result from the presence of only a few worms (Table 1, experiments 3E, 3G.1). The experiments summarized in Table 1 show also that in warm-blooded animals metacercariae may die and begin to disintegrate in lenses that are either normal in appearance (experiment 3L) or severely damaged (experiment 3K). If one of the latter lenses were examined after the metacercariae had died and disintegrated, little trace of the agent causing the disease would remain. Salzer (1907) described the repair occurring in slightly damaged lenses of fish in which strigeid metacercariae had died and disintegrated. A similar study was not made with the diseased eyes in the warm-blooded animals employed in the experiments. That repair of the lens and its return to an apparently normal condition might occur in some of these warm-blooded forms, and even in man, are possibilities.

Until proved a non-existent danger, and in view of the earlier European records,

human parasitization with metacercariae of *D. flexicaudum* can not be considered impossible.

SUMMARY

Cercariae of the strigeid trematode, *Diplostomum flexicaudum*, and other cercariae considered to be probably the same species, were able to penetrate and grow into metacercariae in the lenses of tadpoles, frogs, turtles, chicks, ducklings, mice, rats, guinea pigs, and rabbits. These metacercariae were normal in appearance and behavior and were similar to those developing in the eyes of fishes, the natural intermediate hosts. Metacercariae from the lenses of a frog and a guinea pig were infective for baby chicks, and normal adult worms with eggs were recovered from the bird intestine after one week. Blindness was caused in many of the infected eyes. Certain experiments and an apparently complete lack of records in medical literature suggest that the possibility of these eye flukes causing disease in man is rather remote. However, it remains to be proved that such a danger does not exist.

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A NEW TREMATODE, *DICTYANGIUM CHELYDRAE* (MICROSCAPHIDIIDAE = ANGIODICTYIDAE), FROM THE SNAPPING TURTLE, *CHELYDRA SERPENTINA*

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Looss (1899) designated *Monostoma reticulare* van Beneden, 1859 and *M. proteus* Brandes, 1891 as types of the two genera *Microscapha* and *Baris*, respectively. Since both generic names were preoccupied, Looss (1900) proposed the names *Microscaphidium* and *Deutero-baris* to replace the homonyms and he included the two genera in the subfamily MICROSCAPHIDIINAE. Later, Looss (1902) designated the three species *Microscaphidium linguatula* (Looss, 1899), *Microscaphidium sagitta* (Looss, 1899) and *Microscaphidium parallelum* (Looss, 1901) as types of the new genera *Polyangium*, *Octangium* and *Angiodictyum*. In this paper he included *Microscaphidium*, *Angiodictyum* and *Polyangium* in the subfamily MICROSCAPHIDIINAE while *Octangium* and *Deutero-baris* were designated as types of the subfamilies OCTANGIINAE and DEUTEROBARIDINAE. The three subfamilies were included in a family which he named ANGIODICTYIDAE. Since *Angiodictyum* is not the type of the subfamily to which it belongs, it can not be the type of the family and the name ANGIODICTYIDAE is invalid. In an attempt to rectify the incongruity, Poche (1925) proposed the subfamily ANGIODICTYINAE, with the following explanation, "Den Namen *Angiodictyinae* führe ich an Stelle von *Microscaphidiinae* Looss (1900, p. 605; 1902, p. 699) ein, weil diese Unterfamilie die typische Gattung der Familie *Angiodictyidae*, nämlich *Angiodictyum* Looss, enthält und somit die typische Unterfamilie dieser Familie ist und es mit dem Begriff 'typisch' unvereinbar ist, dass die typische Gattung der typischen Unterfamilie einer Familie eine andere ist als die der Familie." Poche thereby renamed a previously validly named subfamily and ANGIODICTYINAE is a subjective synonym of MICROSCAPHIDIINAE. A discussion of the taxonomic principle involved here and an opinion by Professor C. W. Stiles on it appear in a paper by Stunkard and Nigrelli (1930). Travassos (1922) proposed the name MICROSCAPHIDIIDAE and has used it in his later papers. Although a history of the case shows that it is the only available one, most subsequent authors have accepted the name ANGIODICTYIDAE.

All of these trematodes studied by Looss were from the intestine of marine turtles. In his monographic report, Looss (1902) gave a detailed account of their morphology and recognized for the first time that the vascular channels which ramify through their bodies represent two distinct and independent systems. One set of tubules was identified as a portion of the excretory system, whereas the other was described as a "Lymphgefäßsystem."

Since the monograph by Looss, there have been few additions to knowledge of the group. Odhner (1911) reported a new but undescribed and unnamed species from *Teuthis* sp., a fish from the Red Sea, and asserted that the angiodictyids are amphistomes in which the acetabulum has been lost. The notch at the posterior end of forms such as *Octangium* was regarded as a result of the reduction and dis-

appearance of a terminal sucker. He pointed out that the morphological agreement of *Distomum quadrangulatum* Daday, 1907 with the amphistomes and angiodictyids may be an indication of genetic relationship. Travassos (1922) created the new genus *Parabaris* to receive *P. parabaris*, a species from the intestine of a Brazilian fresh-water fish, *Piaractus brachypomus*. Referring to *D. quadrangulatum*, Travassos, Artigas and Pereira (1928) stated that this species may be congeneric or even specifically identical with *P. parabaris*. Subsequently Travassos (1934) definitely assigned *D. quadrangulatum* to *Parabaris*, and in accord with the suggestion of Odhner, he included the microscaphids, the amphistomes and other supposedly related groups in the PARAMPHISTOMOIDEA. Poche (1925) created a new genus *Nephrobius* for two specimens labelled, "*Monostoma nephrit. ren. Colymb. arctici*" which he found in the Graz collection. Goto and Ozaki (1929) described *Hexangium sigani* from the intestine of *Siganus fuscescens*, a Japanese marine teleost. The most recent contribution is by Price (1937) who created two new genera; *Octangioides* to contain a species from the Mexican fresh-water turtle *Dermatemys mawwii*, and *Hexangitrema* for a species from the black angelfish *Pomacanthus arcuatus*. In a footnote, Price suppressed *Nephrobius* as a synonym of *Polyangium*.

The examination of 79 specimens of *Chelydra serpentina* collected in Louisiana yielded 16 infections and a total of 149 specimens of a new microscaphid trematode for which the name *Dictyangium chelydrae* is proposed. The turtles were large, with shell lengths of 6 to 11 inches. The worms were found only in the initial portion of the large intestine. The number of mature specimens found in individual turtles was always small. The largest number, 36, was found in a turtle dissected in May, 1942. It contained 8 mature and 28 juvenile worms.

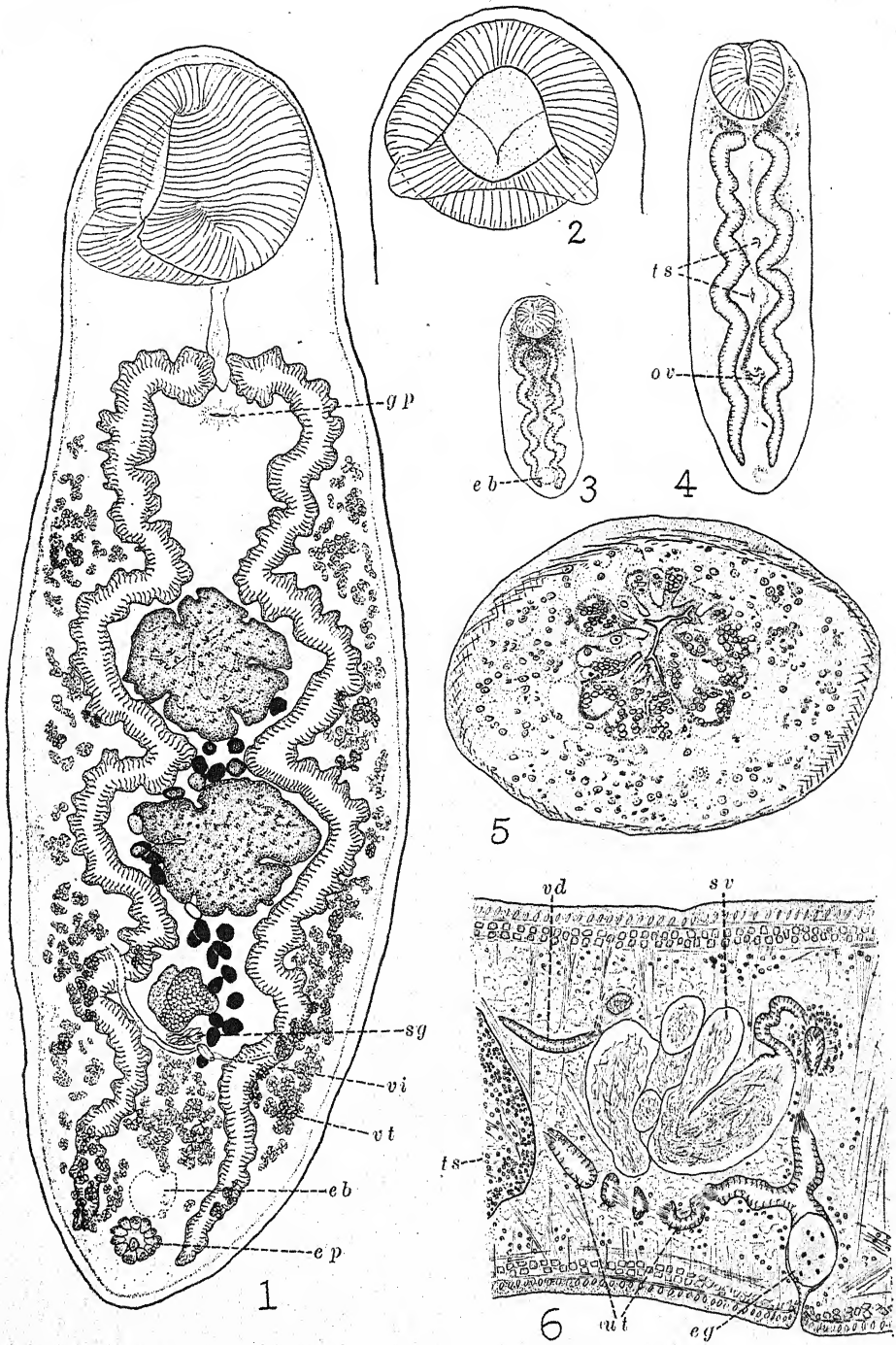
Dictyangium chelydrae n. g., n. sp.
(Figs. 1-6)

The worms are elongate, rounded at the ends, with almost parallel sides. They are flattened, convex dorsally and concave ventrally. On contraction, the anterior and posterior ends bend ventrally, forming an elongate, cupuliform depression. For this reason it is very difficult to secure well extended specimens and whole mounts were made only from worms fixed under the pressure of a cover glass. As they move forward, the worms turn spirally on the long axis. Sexually mature specimens may extend to a length of 6 mm and contract to a length of 2 mm. In width they measure from 0.5 to 1.5 mm. Gravid specimens, fixed, stained and mounted, measure from 2.5 to 4 mm in length and 0.6 to 2 mm in width. In young worms (Figs. 3, 4) the gonads are represented by small clusters of germinal cells, and the dorsal surface of the body is underlaid with diffuse pigment which is most abundant at the level of the intestinal bifurcation and extends posteriad in a V-shaped pattern. There are no cuticular spines, but the musculature of the body wall presents a cross-hatched appearance which suggests a spined cuticula. As in other members of the family, the musculature of the body wall is well developed and bundles of longitudinal fibers are present inside the diagonal layer. The dorsoventral muscles are numerous and very strong.

The anterior sucker is 0.45 to 0.6 mm in diameter, and usually spherical in outline. Details of its structure are shown in Fig. 2. Paired, conical to auriculate, ventrolateral papillae may be protruded as lobes, but there are no retrorodorsal diverticula and the wall of the sucker shows no indication of such evaginations. It is limited by strong external and internal membranes and the fibers are arranged in a definite pattern. Branched fibers extend from the internal to the external membrane and parallel meridional fibers extend around the internal and external faces. In addition, a band of fibers passes around the sucker, dividing it into cortical and central portions, with most of the nuclei situated in the cortical zone. The sucker is anchored to the body wall by protractor and retractor muscles, and small lip-like projections of the body wall may be protruded around the mouth. The oral opening is triangular, wider anteriorly and tapering more or less regularly to a point posteriorly. There is no sphincter and the mouth can not be completely closed. Instead, the sides converge from posterior to anterior, leaving a slit-like aperture with a

triangular opening in front. The absence of a sphincter also imparts a certain flaccidity to the sucker, which under pressure often collapses (Fig. 1). The lumen of the sucker is triangular and tapers retror dorsally to become continuous with that of the esophagus. The anterior end of the esophagus is spanned dorsally by the commissure of the nervous system and it then makes a U-shaped bend, passing ventrally and then dorsally where it communicates with the two lateral digestive ceca. The cuticular lining of the esophagus separates the epithelia of the ceca so that they are not continuous. The posterior end of the esophagus is frequently somewhat dilated, but there is no muscular esophageal bulb, and no appreciable increase in thickness of either the circular or longitudinal coats. The ceca have saccate walls and end blindly near the posterior end of the body. Their course is sinuous but characteristic; they bend laterally leaving wider median areas at the levels of the copulatory organs, each of the testes, the ovary, and the excretory bladder. This pattern is manifest in young specimens (Figs. 3, 4), even before there is any trace of the reproductive organs. Histologically, the longitudinal and circular muscles, reduced in size and number, extend from the esophagus along the walls of the ceca. The epithelium has a deeply staining basal zone in which nuclei are conspicuous but in which cell boundaries are indistinct and the cells bear long cytoplasmic projections, striated in appearance and resembling cilia, which extend into the lumen.

The excretory system is very complicated. It consists of a tubular collecting system which originates at the flame cells, a series of vesicular channels, and a pulsatile bladder. The pore is median, dorsal, near the posterior end of the body. The musculature of the body wall is modified to form a sphincter around the opening and a duct, lined with cuticula, extends ventrad and anteriorly to open into the ventroposterior end of the excretory bladder. The internal end of the duct is provided with a weak sphincter. From the duct, near the external sphincter, a dozen or more small tubules radiate to open into flask-shaped enlargements (Fig. 5) that comprise the "rosette." The tubules are lined with cuticula and the enlargements are more or less completely filled with deeply staining nuclei. Cell outlines were not observed, but open spaces in the enlargements may contain traces of a coagulated material. The bladder has a very thin wall which is much folded when collapsed. It is lined with a low epithelium. In living specimens the bladder and vesicular channels contain a fluid in which fine droplets are suspended, and movement of the droplets facilitates observation and makes it possible to follow the course of the channels. On each side the bladder communicates with a thin-walled, irregularly-shaped sac. The connections were observed first in living specimens and confirmed later by the study of serial sections. From the ventral part of the sac, a main channel passes forward and another backward. The posterior one gives off a branch which passes posteriorly and subdivides to form smaller branches, while the main trunk courses laterally, behind or below the cecum and passes forward along its lateral face. The anterior one gives off a branch which then subdivides, but the trunk turns dorsad to join the main dorsal longitudinal channel which extends forward but also continues backward to partially over-lie the thin-walled sac that opens into the bladder. Actual communication with the sac, although indicated, was not demonstrated. Observation of this region is difficult since the bladder is surrounded by vitelline follicles, the body is opaque, the bladder contracts with explosive suddenness, the bladder may be partially over-lapped by the lateral sacs and these in turn by the intestinal ceca, while the parenchyma is so loose that intercellular spaces may be confused with portions of the excretory system. The vesicular portion of the excretory system consists of the two main trunks on each side of the body, one median and dorsal, the other lateral to the cecum, together with a large number of branches from each of them. The branches may subdivide and usually they end blindly with expanded tips. When collapsed, they are hard to follow in sections. Although the position of the larger branches is relatively constant, there is much variation, especially in the smaller channels. Diverticula from the lateral and median trunks of one side may communicate in the anterior half of the worm and communications between the two sides may occur at the level between the testes, near the bifurcation of the digestive tract, and the channels of the two sides are always united by a loop which passes anterior and dorsal to the sucker. The vesicular channels are lined with a low epithelium and the walls appear to be membranous, although the lumen may expand and contract without any apparent movement of the surrounding tissues. This observation suggests that the vessels have muscular elements in their walls, although changes in their size and shape may be due to contraction of fibers in the adjacent parenchyma. Movement of fluid in the tubules is often rapid and the flow may be in either direction, although eventually the fluid passes backward and out of the excretory pore. The vesicle is well developed in young individuals (Fig. 3), but the channels are so small that it is difficult to trace them. Near the anterolateral margins of the digestive ceca, collecting ducts from the flame cells open into the vesicle. Each duct passes backward and medially, accompanied by a recurrent tubule which joins it in front of the anterior testis. Each collecting duct also receives a branch at the level between the testes, another just posterior to the posterior testis, and



another behind the ovary. These secondary tubules give rise to a very large number of capillaries, each of which ends in a flame cell. The flame cells are small and very numerous; more than 30 were counted on one side between the sucker and the level of the anterior margin of the cephalic testis, but despite repeated attempts it has been impossible to determine the precise pattern of branching in the tubules.

A lymph system, which supposedly is characteristic for the family, is not present.

The testes are large lobed organs, the anterior one situated near the middle of the body and the posterior one a short distance behind it. They are approximately the same size and measure from 0.2 to 0.5 mm in diameter. Usually both are actively functional although vitelloid droplets were observed in the testes of 3 out of 15 worms sectioned and in 2 of 28 mature specimens mounted in toto the anterior testis is degenerate. The vas efferens from the posterior testis arises at its median, anterior, dorsal margin and passes forward and ventrad. It then loops dorsad and near the posterior margin of the anterior testis is joined by the vas efferens from that gonad. The vas from the anterior testis arises at the dorsal margin of the testis and passes posteriad, joining the one from the caudal testis to form the vas deferens which passes forward dorsal to and on the right side of the anterior testis. In front of the anterior testis this sperm duct opens into a large, thin-walled, much coiled seminal vesicle which occupies the region between the anterior testis and the level of the genital pore. The seminal vesicle (Fig. 6) is continued by a thick-walled duct which turns ventrad and opens into the genital atrium. A portion of this duct, about the second quarter of its length, is surrounded by secretory cells. There is no cirrus sac or cirrus.

The ovary is situated slightly to the right of the median line, approximately its diameter behind the posterior testis. It is lobed and measures from 0.1 to 0.2 mm in diameter. The oviduct arises at the dorsal margin, coils mediad and posteriad where Laurer's canal diverges and passes in a sinuous course to open at the dorsal surface of the body. After the origin of Laurer's canal, the oviduct turns ventrad, receives the common vitelline duct, and coils posteriad, dextrad and ventrad where it enters the shell gland and expands to form the ootype. The uterus emerges from the left side of the shell gland, coils backward and then forward on the left side of the ovary, the right side of the posterior testis, the left side of the anterior testis, and then below the seminal vesicle to the genital atrium where it enters behind the opening of the male duct. The atrium (Fig. 6) is shallow and the genital pore is median, ventral, immediately behind the bifurcation of the digestive tract. The vitellaria are voluminous, occupying the extracecal areas behind the genital pore and the median field behind the ovary. Ducts from the two sides converge ventrally and unite behind the ootype to form a vitelline receptacle from which a common duct passes dorsally and dextrad to join the oviduct as described above. There is no seminal receptacle, but the initial portion of the uterus is filled with masses of spermatozoa. The eggs are oval, operculate, and relatively few. Recently extruded eggs in Ringer's solution, without a coverglass, measured 0.10 to 0.11 mm in length and 0.069 to 0.075 mm in width. The ovum is about 0.02 mm in diameter and is located near the opercular end of the egg.

The eggs are passed in the one-celled condition and at laboratory temperatures developed active, ciliated miracidia in one month. Although no life histories have been determined in the family MICROSCAPHIDIDAE, the appearance of young worms

Dictyangium chelydrae n. g., n. sp.

FIG. 1. Type specimen, 3.95 mm long, whole mount, ventral view.

FIG. 2. Anterior sucker, ventral view, to show details of structure.

FIG. 3. Juvenile specimen, same magnification as Fig. 1.

FIG. 4. Juvenile specimen, with rudimentary reproductive organs, same magnification as Fig. 3.

FIG. 5. Cross section, showing details of the "rosette."

FIG. 6. Reconstruction of three sagittal sections, showing genital pore and terminal portions of the reproductive ducts.

eb excretory bladder
eg egg in genital atrium
ep excretory pore
gp genital pore
ov ovary
sg shell gland

sv seminal vesicle
ts testis
ut uterus
vd vas deferens
vi vitelline duct
vt vitellaria

(Fig. 3) recovered from the turtle, suggests that the cercariae are pigmented monostomes. In these young specimens the excretory vesicle has attained its definitive form, although it probably is not developed in the cercaria.

Dictyangium may be distinguished from *Octangium* Looss, *Octangioides* Price and *Hexangitrema* Price by the absence of a posterior median notch, from *Deutrobaris* Looss by the form of the sucker and the absence of rows of ventral glands, from *Parabaris* Travassos by the location of vitellaria and uterine coils, from *Hexangium* Goto and Ozaki by the absence of cirrus sac and location of the gonads, and from *Microscaphidium* Looss, *Angiodictyum* Looss and *Polyangium* Looss by the absence of lymph vessels and differences in the form of the excretory system.

DISCUSSION

Members of the family MICROSCAPHIDIIDAE appear to be limited to tropical or subtropical regions, and infect both marine and fresh-water turtles and fishes. Since the final hosts of *Dictyangium chelydrae* have a much wider distribution than the parasite, it seems probable that the range of the fluke is limited by that of its intermediate host or hosts. Looss found these trematodes only in adult turtles, and since in the present study only large snapping turtles were examined, no information is yet available concerning the size of the turtles when the infection is acquired. Seasonal variation in egg production and occasional degeneration of the gonads were discussed by Looss (1902) and other authors, but the final explanation of these phenomena will probably not be forthcoming until the life history is known.

The family MICROSCAPHIDIIDAE (ANGIODICTYIDAE) as constituted by Price (1937) contained nine genera, to which *Dictyangium* should be added. These trematodes, although constructed on the same morphological pattern, show marked structural variation. The posterior end of the body may be rounded or manifest a distinct notch. With the exception of *Distomum quadrangulatum* Daday, 1907, they are monostomes, and the inclusion of *D. quadrangulatum* in the genus *Parabaris* by Travassos (1934) is questionable. Daday (1907) described and figured an acetabulum, 0.13 mm in diameter, situated immediately behind the genital pore, and if the original account is correct, (its accuracy was questioned by Odhner, 1911) the specimen described by Daday is probably not congeneric with *Parabaris parabaris*. In the family, the anterior sucker may be simple, may bear ventrolateral muscular lappets which recall those on the acetabulum of *Zygocotyle*, or may possess retrodorsal evaginations similar to those of many amphistomes. The esophagus may or may not have a posterior muscular thickening. The digestive ceca may extend almost to the posterior end of the body or terminate anterior to the gonads. The anterior sucker of the microscaphids is probably homologous with that of the amphistomes and of the pronoccephalids, but there is much difference of opinion whether it is the equivalent of the oral sucker or the pharynx of other trematodes. Looss (1902) reviewed the subject and argued that this anterior sucker is an oral sucker and that it is not homologous with the pharynx. The muscular thickening at the posterior end of the esophagus he designated as a pharynx. This opinion was accepted by most subsequent helminthologists until Näsmark (1937) reopened the question. He stated, p. 354, "A detailed anatomical or histological comparison shows that the 'oral sucker' of the Paramphistomides is in principle constructed on the same anatomical scheme as that found in the pharynx bulbosus of the

rhabdocoelous Turbellarians, and the pharynx of the monogenous Trematodes." He regarded the "oral sucker" of other trematodes as a secondarily acquired organ, situated in front of the pharynx, and derived from the walls of the prepharyngeal part of the oral cavity. Accordingly, he stated that the amphistomes have no "oral sucker" and the anterior sucker was designated as the pharynx. Willey (1941) regarded the evidence of Näsmark as incomplete and not convincing. The problem is a difficult one, requires the consideration of extensive data, and is actually outside the scope of the present paper. Accordingly, to avoid predilection, the organ is here described as the anterior sucker. Associated with the problem of the oral sucker is that of the esophageal enlargement or bulb, situated at or near the posterior end of the esophagus. It was termed "pharynxbulbus" by Odhner (1911) who stated, "Ich verwende diese Bezeichnung, weil es mir doch nicht so ganz sicher erscheint, dass es sich hier um ein dem gewöhnlichen Distomenpharynx homologes Organ handelt. Auch wenn es so wäre, könnte ubrigens der ziemlich verschiedene Bau einen besonderen Namen rechtfertigen; der Oesophagus müsste aber dann konsequenterweise als Präpharynx bezeichnet werden." This structure, usually but not always present in amphistomes and microscaphids, consists essentially of a thickening of the muscular wall, especially the circular fibers of the region. As noted by Odhner and subsequent authors, its structure is very different from that of the pharynx of other flatworms.

Another problem is presented by the excretory and lymph vessels in members of the family. The vesicular portion of the excretory system may consist of from two to eight branching longitudinal vessels and a lymph system may or may not be present. There is still uncertainty concerning the relations of the excretory and lymphatic channels. These vessels were regarded as portions of the excretory system until Looss (1902) recognized the lymph vessels as a distinct and independent system. Earlier accounts were reviewed and new observations on lymph systems were reported by Willey (1930a, 1933). Willey (1930b) pointed out that the excretory system of *Hexangium sigani* as described by Goto and Ozaki (1929) differs from that of previously described members of the ANGIODICTYIDAE, while in structure and distribution it recalls the lymph vessel system of these trematodes. He suggested the possibility that the three pairs of longitudinal vessels in *Hexangium* may be lymph channels. Later, Willey (1935) described the excretory system of *Typhlocoelum cucumerinum*. On each side there are three main longitudinal vessels which unite posteriorly and a common stem communicates with the excretory bladder, an arrangement similar to that in *Hexangium*. The vessels give off numerous branches that form a reticulum. Histologically the vessels have membranous walls and they contain a granular coagulum in which lymphocytes are suspended. He stated, "Many features of the excretory system of *Typhlocoelum cucumerinum* are similar to those found in the lymph system of amphistomes. The intimate association with the intestine, the cuticular walls of the vessels, the granular coagulum in the lumen, and the presence of numerous cellular elements comparable to the 'lymphocytes' strongly suggest a lymph system. However, since the vessels communicate with the excretory vesicle, the lumen of which contains the same elements, and since no other excretory organs are to be found, the structure must be interpreted as an excretory system." Ozaki (1937) redescribed the excretory system of *Hexangium sigani*; in addition to the three pairs of excretory vessels, he

reported the presence of three pairs of lymph channels in this species. Although Looss (1902) described a lymph system in all of the microscaphids studied by him and stated that such a system is characteristic of the family, lymph vessels were not observed by Price in *Hexangitrema pomacanthi* and they are absent in *Dictyangium chelydrae*. A detailed study of the development of the lymph system in those species in which it occurs should provide the basis for a correct interpretation of the relation between excretory and lymphatic vessels.

SUMMARY

The family name ANGIODICTYIDAE Looss, 1902 is invalid and should be suppressed in favor of MICROSCAPHIDIIDAE Travassos, 1922. *Dictyangium chelydrae* n. g., n. sp., a member of the family, is described from *Chelydra serpentina* in Louisiana. Morphological similarities between the microscaphids and amphistomes are discussed. The absence of a lymph system in *D. chelydrae* necessitates a change in the diagnosis of the MICROSCAPHIDIIDAE. Relations between the lymph and excretory systems are considered.

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PRELIMINARY OBSERVATIONS ON THE EFFICACY OF A PRODUCT
FROM OIL OF ROSE GERANIUM FOR THE REMOVAL
OF INTESTINAL PARASITES FROM DOGS

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In preliminary tests of several substances for anthelmintic action, one, a product from oil of rose geranium, gave promising results in dogs, both with respect to its anthelmintic action and its apparent lack of toxicity.

The product was prepared from a sample of oil of rose geranium of domestic origin furnished by the Bureau of Plant Industry. Geraniol was removed by means of calcium chloride, and the remaining part was treated with phthalic anhydride. The acid phthalate thus formed was extracted with sodium carbonate. The sodium acid phthalate was saponified with potassium hydroxide and the liberated alcohol separated by steam distillation. The product was then distilled under reduced pressure. It had a boiling point of 111 to 117° C, $p = 12$ mm; density at 25° C (g per cc), 0.8609; refractive index $n_{\frac{25}{D}}$, 1.4557; optical rotation (α) $\frac{20}{D}$, 0°.

When analyzed by the formylation method, the substance had a citronellol content of about 92 per cent. The remaining 8 per cent presumably consisted of the alcohols found in oil of rose geranium, other than geraniol.

So far as could be determined, the literature contains no reference to the anthelmintic action of the product used in these studies, although Rico (1929, Compt. Rend. Soc. Biol., Paris, 102: 218) reported that a substance regarded by him as geraniol gave encouraging results against hookworms when administered to two dogs.

The present tests on the anthelmintic action of this compound were conducted at the station of the Zoological Division, Beltsville Research Center, Beltsville, Maryland. The drug was administered in gelatin capsules to dogs after a fasting period of 18 hours. When a purgative was used it was given from 1½ to 2 hours after treatment, and consisted of one ounce of castor oil. The feces were examined daily for parasites in the usual manner. When no additional worms were eliminated, the animals were destroyed and the intestinal tracts examined for the presence of parasites. The usual length of time from the treatment until necropsy was 4 days.

The results obtained are shown in Table 1. Two species of ascarids were present in these dogs, *Toxascaris leonina* and *Toxocara canis*, both of which were removed by comparatively small doses of the drug. Of the two species of hookworms that were present, *Ancylostoma caninum* and *Uncinaria stenocephala*, the latter was removed by small amounts of the drug while *Ancylostoma caninum* appeared to be more resistant and required larger doses. In order to effect the removal of whip-

worms, *Trichuris vulpis*, it was necessary to increase the dose rate even more. The use of a purgative did not appear to increase the efficacy or safety of the drug.

From the limited information obtained, dosages of from 0.5 cc to 0.7 cc per pound of body weight seemed to be adequate for the removal of ascarids and hookworms from dogs, although the data are insufficient to recommend this dose rate for other than experimental purposes. At no time during the course of these experiments were any clinical symptoms of toxicity evidenced by the dogs treated, nor were any lesions observed at autopsy that could be attributed to the drug. Moreover, unlike the majority of anthelmintic drugs, there is not the factor of an un-

TABLE 1.—Data on anthelmintic tests in dogs with a product obtained from oil of rose geranium

Dog No.	Weight (pounds)	Amount of drug given (also purgative if used)	Parasites passed	Parasites found post mortem	Efficacy (per cent)
60	22	5 cc	3 ascarids	0 ascarids	100
			13 hookworms	2 hookworms	87
62	19	5 cc plus one ounce of castor oil	13 ascarids	0 ascarids	100
			29 hookworms	9 hookworms	76
51	12	5 cc	21 whipworms	200 whipworms	10
			4 ascarids	0 ascarids	100
52	15	5 cc	3 hookworms	0 hookworms	100
			2 ascarids	0 ascarids	100
			1 <i>Taenia hydatigena</i>	0 tapeworm	100
61	12	1 cc	2 ascarids	0 ascarids	100
			1 hookworm	0 hookworms	100
53	10	2.5 cc	5 ascarids	0 ascarids	100
			2 hookworms	0 hookworms	100
			1 whipworm	3 whipworms	25
58	18	17.5 cc plus one ounce of castor oil	1 ascarid	0 ascarids	100
			233 hookworms	0 hookworms	100
			247 whipworms	116 whipworms	68
43	26	20 cc plus one ounce of castor oil	2 hookworms	0 hookworms	100
			12 whipworms	29 whipworms	30
48	27	25 cc plus one ounce of castor oil	1 ascarid	0 ascarids	100
			3 hookworms	0 hookworms	100
66	12	15 cc	1 hookworm	0 hookworms	100
			3 whipworms	24 whipworms	20
				8 <i>Dipylidium caninum</i>	0
69	25	12.5 cc plus one ounce of castor oil	11 ascarids	0 ascarids	100
			89 hookworms	0 hookworms	100
				30 whipworms	0
70	20	10 cc	4 ascarids	0 ascarids	100
			10 hookworms	0 hookworms	100
			2 whipworms	20 whipworms	10
				46 <i>Dipylidium caninum</i>	0
46	40	5 cc plus one ounce of castor oil	3 hookworms	0 hookworms	100
67*	30	7.5 cc	none	none	?
68*	25	12.5 cc	none	none	?

* Although these dogs passed no worms after treatment and none were found on postmortem examination, hookworm ova were present in the feces before treatment and were absent four days afterwards.

pleasant odor; rather this preparation possesses a fragrance of rose perfume. It is possible that this drug may compare favorably with other anthelmintics for dogs, and may prove useful as a possible substitute for the increasingly scarce chlorinated hydrocarbons that are now widely used.

This product is not widely available at present, but could probably be made so if further experience justified its use. At a dose rate of 0.5 cc per pound of body weight it failed to remove ascarids satisfactorily from one pig; other than this single trial no data have been obtained on its possible usefulness for the removal of parasites from other host animals.

Summarizing the data herein, it will be noted that in doses varying from 1 to 25 cc per dog, a substance consisting largely of citronellol removed 100 per cent of 46 ascarids from 10 dogs, 97 per cent of 400 hookworms from 12 dogs, and 40 per cent of 708 whipworms from 7 dogs.

RESEARCH NOTES

THE RELATIVE INCIDENCE OF BLOOD PARASITES IN SOME BIRDS FROM GEORGIA

The data reported here are the results of a survey dealing with the relative incidence of blood parasites in various species of wild birds trapped in Georgia during the last four years.

The birds were trapped throughout the year, with all but 8 being taken in Bulloch County. Diagnosis for the presence of parasites was based upon a 10-minute examination of a single stained blood smear.

Results: In 275 birds belonging to 14 families and 23 species 20% of the total number of birds examined were infected with one or more genera of parasites. Mixed infections were observed in 16 cases, giving a total of 84 infections in the 55 infected birds. The percentage of birds infected with each type of parasite was as follows: *Plasmodium*, 4%; *Haemoproteus*, 9.5%; *Leucocytozoon*, 6%; *Trypanosoma*, 4%; and microfilariae, 3%. The species of infected birds, the parasites they harbored, and the number of birds in each species microscopically negative are given in Table 1.

Experimental: It has been shown by Manwell and Herman (1935, Am. J. Trop. Med. 15: 661) that infections of *Plasmodium* are more readily detected by inoculating blood into clean canaries than by simply examining blood smears. In the present study 39 inoculations have been made into microscopically negative birds of the same species as the birds being examined. This method revealed an incidence of 31% among birds not showing patent plasmodial infections. The infections encountered by using this technique, along with symbols to indicate the new host records for the various parasites are also given in Table 1.

Multiple infections were encountered in 17 birds; these are summarized in the following tabulation in which the abbreviations used are *Haemoproteus* (H), *Leucocytozoon* (L), *Plasmodium* (P), *Trypanosoma* (T), microfilariae (M). H, L—1 dove, 1 blue jay, 1 vesper sparrow; H, M—2 blue jays; H, T—1 cardinal; H, T, M—1 blue jay; H, L, M—2 blue jays; H, P, L—1 blue jay; H, L, T, M—1 blue jay; P, L—1 cowbird; P, T—1 cowbird, 1 brown thrasher; P, L, M—1 blue jay; P, T, M—1 blue jay; P (2 species)—1 cardinal.

No blood parasites were found in 11 species of birds in which from 1 to 3 specimens were examined.

Comparison with results of other surveys: Several surveys of avian blood parasites have been made in widely separated localities, and as the number of these is extended it should be possible to describe the geographic distribution of the various species of blood parasites. The species of birds and the number of specimens in each species have varied greatly. Since it is readily apparent from these studies that the various species of birds show much variation in both the kind and number of blood parasites, the results may best be compared on a basis of the species of hosts examined rather than upon the total number of parasites encountered in all birds examined. The comparisons that can be made are not entirely satisfactory inasmuch as the results are subject to the distorting effects of incomplete sampling and possibly of variations in the seasons during which the smears were collected. Comparisons are made with the following studies: Huff (1939, J. Am. Vet. Med. Assn. 44: 615) mainly in the Middle West, Manwell and Herman (1935, Am. J. Trop. Med. 15: 661) in upper New York State, Herman (1938, Tr. Am. Micr. Soc. 57: 132) on Cape Cod, and Wetmore (1940, J. Parasitol. 27: 379) in the District of Columbia.

English sparrows: Huff (in 125 specimens) found *Plasmodium* in 10%, with 4% of the total number harboring *P. cathemerium*; Manwell and Herman (in 245 birds) found *Plasmodium* in 3%, all of which were *P. relictum*. Thus *P. cathemerium* was the predominant species of *Plasmodium* in the birds examined by Huff but was not encountered in the studies made in New York and Georgia.

White-throated sparrows: Huff observed *Haemoproteus* in 40% of the 21 birds examined; Wetmore found *Haemoproteus* in 31% and *Leucocytozoon* in 15% of 19 birds examined. Neither of these parasites was seen in 16 specimens taken in Georgia.

Starlings: In the various surveys only *Plasmodium* has been recorded and these infections have been encountered rarely. Manwell and Herman found one infection in 120 birds; Wetmore found two infections in 32 birds; and Huff examined 11 birds with negative results.

Blue jays: This bird is evidently one of the most commonly infected. Huff observed *Haemoproteus* in 44% (43 examined); Wetmore found *Haemoproteus* in 17%, *Leucocytozoon* in 14%, *Plasmodium* in 3% (29 examined). In the Georgia study 27 birds (trapped mainly in the spring) harbored a total of 40 infections with *Haemoproteus* occurring in 52%; the infections also included trypanosomes and microfilariae which were not reported in the studies by Huff and by Wetmore.

TABLE 1.—Species of birds infected with one or more blood parasites; also number of individuals examined and number of individuals uninfected of these species

Hosts		Parasites										Number examined	Number negative
Family and scientific name	Common name	Plasmodium						Haemoproteus	Leucocytozoon	Haemosporidia (Genus undetermined)	Trypanosoma	Microfilariae	
		circumflexum	elongatum	hexamerum	relatum	vancouveri	Undetermined						
Columbidae		2	2	3
Zenaidura macroura eurolinensis	Mourning dove	6
Columbidae	Black-throated blue warbler	2*	1	0
Columbidae	Blue jay	1*	..	1*	1†	14	7	1	6	8	27
Columbidae	Cardinal	1*	1*	..	1*	2	1	..	2	..	7
Columbidae	Red-eyed towhee	1*	1	4
Columbidae	White-throated sparrow	1*	1†	4
Columbidae	Cowbird	2†	..	2†	..	3	1	12	..	14
Columbidae	Vesper sparrow	1*	6	2*	1	60
Columbidae	Mockingbird	2*	2*	..	1	1	..	19
Columbidae	Brown thrasher	1	10
Columbidae	English sparrow	2	..	1	6
Columbidae	Starling	38
Totals (exclusive of experimental results)		11	11	26	16	4	13	9	245	190			

* New host record.

† Discovered by subinoculation.

Vesper sparrows: Herman examined 31 specimens during the summer months and found only a single infection of *Haemoproteus* and of *Plasmodium*; while approximately the same number of birds from Georgia examined during the spring showed a greater total number of infections, including *Leucocytozoon* and *Trypanosoma*.

Brown thrashers: Huff found *Haemoproteus* in 29 of 48 birds examined; Wetmore observed this genus in 5 of 12 birds examined; while 9 specimens in the present study were negative.

Cowbirds: Herman examined 378 birds during the summer months with the following results: *Plasmodium* in 14, *Leucocytozoon* in 2, *Plasmodidae* in 3. These results differ from those of the present (in which all the cowbirds were examined during the winter) in a higher incidence of *Plasmodium*, a lower incidence of *Leucocytozoon*, and the absence of *Trypanosoma*.—PAUL E. THOMPSON, Georgia Teachers College.

OPECOELINA PHARYNMAGNA N. SP. (TREMATODA) FROM THE CHINA ROCKFISH

On August 8, 1938, a single specimen of a trematode of the genus *Opecoelina*, family Allocreadiidae, was found in the dissecting dish containing the stomach and intestine of a China rockfish, *Sebastes nebulosus*. The host was taken from the Pacific Ocean, near Fort Ross, California.

The genus *Opecoelina* was erected by Manter (1934, Carnegie Inst. Wash. Pub. 435, Papers Tortugas Lab. 28: 257-345), based upon the descriptions of two new species, *Opecoelina scorpaenae* and *O. helicoleni*. A third species, *O. theragrae*, was added to the genus by Lloyd (1938, J. Parasitol. 24: 103-133). The specimen from the China rockfish appears to represent an undescribed species. For this trematode, the name *Opecoelina pharynmagna*, is proposed.

Opecoelina pharynmagna n. sp.
(Fig. 1.)

Body smooth, elongate, tapering at both extremities; length 3.6 mm. Body length can be separated into four approximately equal portions or units. First unit extends from oral sucker to posterior border of acetabulum; second unit extends to anterior margin of ovary and contains uterus; third unit extends to region just posterior to testes and con-

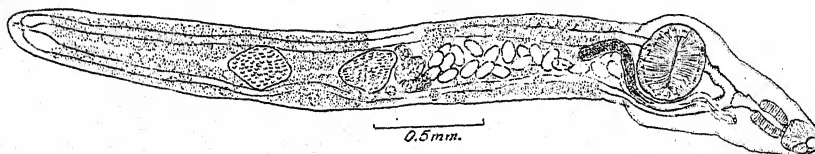


FIG. 1. *Opecoelina pharynmagna* n. sp., ventral view.

tains the gonads; fourth unit extending to posterior end of body, occupied largely by posterior vitellaria.

Greatest width, 0.53 mm through acetabular region. Behind acetabulum body constricts abruptly; at this point body width measures 0.4 mm; from this point lateral margins remain parallel to region of ovary and then gradually taper to posterior extremity.

Oral sucker terminal, mouth ventral, length 0.179 mm, width 0.162 mm; acetabulum wider than long, width 0.36 mm, length 0.3 mm. Prepharynx very short; pharynx nearly as large as oral sucker (0.136 mm \times 0.125 mm); esophagus shorter than pharynx. Intestinal bifurcation approximately 0.14 mm from anterior border of acetabulum; ceca unite in posterior end of body to form short rectum. Anal opening could not be ascertained with certainty; due to expanded character of ceca in this region, it was not, at first, recognized that union existed.

Testes elongate, roughly oval, tandem, approximately 0.27 mm long and 0.185 mm wide. Ovary, four lobed, its posterior margin approaching anterior testis, its anterior margin marking equator of two body halves. Seminal receptacle and yolk reservoir dorsal, and to left of ovary; small portion of seminal receptacle overlapping margin of ovary. Laurer's canal present and to left of mid-line. Vitellarian follicles large and round, extending from point just back of acetabulum, to posterior tip of body, filling all available space, with exception of small area at ovarian level. Cirrus pouch long, sinuous, passing to the left of the acetabulum and ending in the midline, 0.256 mm

behind acetabulum. Seminal vesicle enclosed by cirrus sac. Genital pore to left and slightly anterior to bifurcation of ceca. Uterus confined to intracecal area between ovary and acetabulum. Eggs measure 77-92 microns \times 43-52 microns.

Host: *Sebastodes nebulosus* (Ayres).

Habitat: Stomach or intestine.

Locality: Fort Ross, California.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 36866.

Opecoelina pharynmagna presents many characteristics similar to the other members of the genus. In body size and form it is very similar to *O. helicoleni* Manter and *O. theragrae* Lloyd. It differs from all the other species in having a very large pharynx, which is nearly equal to the size of the oral sucker, and a much shorter oesophagus. Like *O. theragrae*, the seminal vesicle is enclosed in the cirrus pouch, whereas an external seminal vesicle is present in *O. helicoleni* and *O. scorpaenae*.—R. F. ANNEXEAUX, *Animal Pathology Laboratory, California State Department of Agriculture*.

A REDESCRIPTION OF *CONTRACAECUM HABENA* (LINTON, 1900) LINTON, 1934

Numerous ascarids collected from *Opsanus tau* at the mouth of the Patuxent River in June, 1941, and sent to the writer for identification, proved upon comparison with the type specimens of *Ascaris habena* Linton, 1900, to belong to that species. Linton originally described this worm from *Opsanus tau* at Wood's Hole, where he reported it to be of frequent occurrence. In 1934 (James Johnstone Mem. Vol., Liverpool) he referred it to its correct genus, *Contracaecum*. Since Linton's description is inadequate, the following redescription is given:

Contracaecum habena (Linton, 1900) Linton, 1934

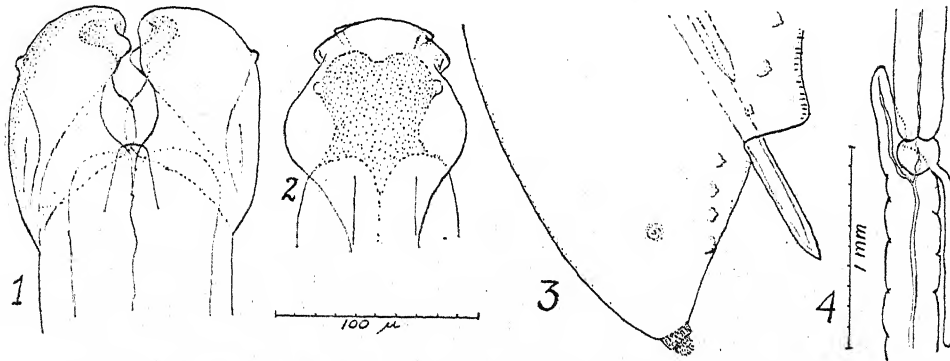
Body tapering anteriorly, reaching greatest diameter near posterior end. Cuticle finely and inconspicuously striated. Greatest diameter through lips 100 to 160 μ , about equal to length of latero-ventral lips; latero-ventral lips each have three interlocking rounded lobes situated one behind the other, the middle one, antero-posteriorly, being most prominent. Dorsal lip slightly shorter than latero-ventral lips, about 90 to 150 μ long; greatest width, slightly posterior to middle, about 10% less than length; an inner flap, forming anterior margin, rounded in front and with two pairs of lateral angles, one pair distal to the other. Pulp of dorsal lip forms two rounded lobes in front, with the papillae situated on the lateral curvatures. Interlabia 30 to 40 μ long, 30 μ wide at base, 15 μ at tip. Esophagus 4.6 to 7.8 mm long, 20 to 25% of length of body. Nerve ring 450 to 600 μ from anterior end. Esophageal ventriculus slightly broader than long, and a little broader than terminal portion of esophagus, measuring about 150 to 180 μ in length and 200 to 250 μ in width. Esophageal appendage about 740 to 970 μ long; intestinal cecum always shorter and less slender, 320 to 530 μ long. Tail in both sexes covered at tip by minute spines.

Male 16 to 30 mm long; maximum diameter 420 to 650 μ . About 24 pairs of small preanal papillae extending for distance of 0.8 to 1.2 mm anterior to cloaca; posterior 15 to 18 of them more ventral in position than the more anterior ones. Papillae near cloaca spaced about 20 to 30 μ apart, the more anterior ones about 60 to 100 μ apart. 4 pairs of ventral postanal papillae; last one largest, situated slightly beyond middle of length of tail and more ventral than others. 1 pair of laterally placed postanal papillae. Body diameter abruptly narrowed behind cloaca; tail 110 to 120 μ long, its tip provided with a spiny, retractile, button-like structure. Spicules 2.7 to 4.2 mm long, about 11% to 15% of body length.

Female 23 to 42 mm long. Maximum diameter 0.58 to 1 mm (1.5 according to Linton). Vulva 38% to 40% of body length from anterior end. Tail 240 to 250 μ long, with retractile, spiny tip.

The species of *Contracaecum* occurring as adults in fishes, unlike those in birds, are strikingly variable with respect to the absolute and relative lengths of the intestinal cecum and the esophageal appendix; whereas in the bird species the intestinal cecum is always long and invariably exceeds the esophageal appendix in length, in the fish species the reverse is true in many instances, and the intestinal cecum may be greatly reduced. The present species belongs with a group (*auctum*, *cornutum*, *fabri*, *habena*, *legendrei*, and *trichiuri*) in which the cecum is only 0.2 to 0.6 mm long, and is exceeded in length by the appendix in all except *auctum*, in which the two diverticula have about the same length. In *cornutum*, *fabri* and *legendrei* the cecum is reduced to a pocket little longer than it is wide, whereas in *auctum*, *habena* and *trichiuri* it is between 0.3 and 0.6 mm long.

These three species are also characterized by having the esophageal appendix less than 1 mm in length. *C. habena* has a relatively longer esophagus and longer spicules than any other species of *Contracaecum* from fishes. Within the group characterized by a short intestinal cecum it most nearly resembles *auctum* in the shape of the lips, and resembles that species in having a spiny tail. Stefanski (1936) reported a *Contracaecum* from *Trachinus draco* which he doubtfully referred to *auctum*, pointing out that it differed from the description of that species as given by Schneider



FIGS. 1 to 3: *Contracaecum habena* (Linton, 1900): 1, latero-ventral lips and interlabium; 2, dorsal lip; 3, posterior end of male. Fig. 4. Region of junction of esophagus and intestine, showing ventriculus, esophageal appendix and intestinal cecum.

(1866) in having prominent cervical alae (a feature shared by *C. trichiuri*) and symmetrical latero-ventral lips. *C. habena* does not correspond with Stefanski's "*auctum*" in these respects, but it can be differentiated from Schneider's meager description of *auctum* only by its narrower dorsal lip with its differently shaped pulp.

Of the three specimens of *Ascaris habena*, two females and one male (U. S. Nat. Mus. Helm. Coll. No. 6533), which Linton designated types of this species, the male is hereby designated the lectotype.—ASA C. CHANDLER, *The Rice Institute, Houston, Texas*.

A CASE OF CANINE STRONGYLOIDIASIS IN TEXAS

According to a review of the literature by Augustine (1940) cases of natural canine strongyloidiasis are rare, having been reported by Fülleborn (Arch. f. Schiffs-u. Tropen-Hyg. 5: 26-80) from China; Ware (1923, J. Comp. Path. and Therap. 36: 108) from India; Faust (1933, Am. J. Hyg. 18: 114-132; 1935, Arch. Path. 19: 769-806) from New Orleans; Whitney (1936, Vet. Med. 31: 104-105) from eastern United States (the correctness of which is questioned); Augustine and Davey (1939, J. Parasitol. 25: 117-119) from Boston; and Lucker (1942, Vet. Med. 37: 128-137) from Atlantic coast states. On the basis of geographic distribution and epidemiology, and of very limited cross-infection experiments, Augustine concluded that the dog strain is distinct from the human and should be recognized as *Strongyloides canis* Brumpt, 1922.

On Feb. 2, 1942, a stool from a 5-months-old cocker spaniel, consisting of a large amount of mucus and some blood, was found to contain numerous eggs of *Strongyloides* in various stages of development, together with a few hatched larvae. The dog was reported to have had dysenteric stools for several weeks, and continued to have them. In fresh stools only unhatched eggs were found. In cultures only direct development was seen. Five thousand filariform larvae were inoculated by subcutaneous injection into each of two dogs, 2500 into a rabbit, 2000 apiece into a rat, a guinea pig, and two young cats, and 800 into a mouse. None of the rodents ever showed any infection; this conforms with results obtained by Augustine and Davey (l.c.) and indicates that the dog parasite is not a rodent strain in an abnormal host. Cultures from both of the dogs were positive on the 11th day; one of the dogs escaped a few days later, but the other remained infected for about 10 weeks and then became negative, in spite of several further injections of filariform larvae derived from this dog's cultures. One of the cats produced positive cultures on the 15th and 18th days after infection, but became negative on the 21st day and thereafter.

The naturally infected dog was treated with 1 cc of CaCl_2 on Feb. 10, whereupon the number of eggs and larvae became greatly reduced, and the stools were much better, but on Feb. 17 a large amount of mucus and blood was passed again. Early in March the dog was given a course of treatment with $\frac{1}{2}$ gr, 1-hour Seal-Ins tablets of gentian violet at the rate of two a day for seven days, which was well tolerated. For about three weeks after treatment the dog had fairly normal

stools, but then passed another dysenteric one; fecal examination showed a small number of *Strongyloides* eggs. Another 8-day course of gentian violet was given. Again the dog's stools improved, and a week after conclusion of treatment no *Strongyloides* eggs could be found, nor had they reappeared two months later. It seems probable that the treatment given was helpful in terminating the infection, but spontaneous recovery of dogs has been reported, so this conclusion must be accepted with some reservation.—ASA C. CHANDLER, *Rice Institute, Houston, Texas.*

NOTES ON THE ADULTS OF *PROTOSTRONGYLUS COBURNI* IN THE LUNGS OF WHITE-TAILED DEER

In a previous study of tissue changes in the lungs of white-tailed deer accompanying natural infections with *Protostrongylus coburni*, no adult worms were encountered in the gross and microscopic examination of the lungs of 20 deer, 14 of which showed infection by the presence of eggs and first stage larvae (Goble, 1941, *J. Wildlife Management* 5: 141-158). No statement concerning the host reaction to the adults could be made but the belief was expressed that the major pathology in certain *Protostrongylus* infections was due to the presence and activity of eggs and larvae rather than to the presence of adults. This opinion has since been supported by examination of the material described below.

During the year ending November 1, 1939, a number of white-tailed deer (*Odocoileus virginianus*) came to autopsy at the Laboratory of Wildlife Pathology, Delmar, N. Y. These may be advantageously treated in two groups. One group consisted of 150 essentially normal, healthy deer which died of various traumatic causes. The other group consisted of 26 deer found dead or dying in a winter deer yard which has been under study for some years in an attempt to arrive at understanding of winter losses in the Adirondack mountains. The autopsies were performed by E. L. Cheatum, Pittman-Robertson pathologist, whose report on the incidence of lungworms in New York deer is in preparation. Following gross examination a slice of tissue 30 mm thick was taken from the diaphragmatic lobe of each lung and fixed in 10% formalin. Routine microtechnique furnished 10 micron sections stained in hemalum and eosin.

Microscopic examination of sections from the lungs of the 150 deer of the first group (normal deer, killed by trauma) revealed infection with *Protostrongylus* in 30%, as evidenced by the presence of eggs and larvae, but adult worms appeared in only 1.3% (2 deer). Similar examination of the material from 26 deer of the second group (found dead or dying) revealed eggs and larvae in 100% and adults in 27% (7 deer). This group contained 18 fawns, in 7 of which adult lungworms appeared and 8 adults in which no lungworms were found. Cheatum's records on over 700 deer autopsies indicate that the incidence of *P. coburni*, obtained by examination for eggs and larvae, is over twice as great in the Adirondacks as it is in the rest of the state. In view of the difficulty encountered in finding adults, the difference in the findings in the above two groups of deer would seem to indicate that in addition to a higher incidence of lungworm infection among the winter-killed deer there was a larger worm load per animal.

It was consistently apparent from the sections showing adult worms in situ that the tissue changes present were not attributable to the presence of the adult lungworms. This is in sharp contrast to the intensive reaction sometimes induced by the arrival of fourth stage larvae in the lung from the blood stream and to the extensive lymphocytic infiltration and septal hyperplasia provoked by the presence of segmenting ova and newly hatched larvae. The fact that the pathological picture in the lungs of two deer, which were shot and immediately autopsied, was the same as in those which died or were killed and sent to the laboratory for autopsy, is regarded as evidence that there was no post-mortem migration of the adult lungworms.—FRANS C. GOBLE, *Wildlife Research Center, N. Y. State Conservation Dept., Delmar, New York.*

NEW HOST RECORDS OF NEMATODES FROM MUSTELIDAE (CARNIVORA)

During the past two years the writer had the opportunity to autopsy 16 weasels [7 *Mustela c. cicognani* (short-tailed weasel) 5 *M. longicauda spadix* (long-tailed weasel), 2 *M. r. rixosa* (least weasel) 2 *M. frenata noveboracensis* (New York weasel)], 30 ranch-raised minks and 8 wild minks (*Mustela vison mink*), 8 skunks [5 *Mephitis hudsonica* (northern plains skunk) 3 *M. m. avia* (Illinois skunk)], 4 badgers (*Taxidea t. taxus*), and 3 martens [2 *Martes a. americana* (American marten) and 1 *M. p. pennanti* (fisher)] which were submitted or collected for examination. Some of the parasitic findings represent new host occurrences so that the results seem worthy of record.

Physaloptera maxillaris was found in the stomach of 3 short-tailed weasels (*Mustela c. cicognani*), 1 least weasel (*M. r. rixosa*) and 1 long-tailed weasel (*M. longicauda spadix*), all new host records for this nematode.

In a previous paper the writer (1941, *Proc. Helm. Soc. Wash.* 8: 23-30) listed the following

North American hosts of *P. maxillaris*: Mustelidae: *Spilogale putorius* (spotted skunk), *S. g. gracilis* (canyon spotted skunk), *S. g. saxatilis* (Great Basin spotted skunk), *S. interrupta* (prairie spotted skunk), *Mephitis mephitis* (Canada skunk), *M. m. mesomelas* (Louisiana skunk), *M. m. nigra* (eastern skunk), *M. m. avia* (Illinois skunk), *M. elongata* (Florida skunk), *M. o. occidentalis* (California skunk), *M. hudsonica* (northern plains skunk), *M. macroura milleri* (northern hooded skunk), *Conepatus mesoleucus venaticus* (Arizona hog-nosed skunk), *Mustela vison mink* (common mink), *M. v. vulgivaga* (southern mink), *Taxidea t. taxus* (common badger), *T. t. berlandieri* (Mexican badger), and *T. t. neglecta* (western badger). Procyonidae: *Procyon l. lotor* (eastern raccoon) and *P. l. elucus* (Florida raccoon). Foreign hosts include: Mustelidae: *Mephitis chinche* (Brazilian skunk) Brazil, *M. suffocans* (bare-nosed skunk) Brazil, *Mydaus* sp. (Borneo badger) Borneo; Procyonidae: *Procyon cancrivora* (crab-eating raccoon) Europe, Trinidad, *Nasua narica* (coatimundi) Brazil.

Later, the writer (1941, Ibid. 8: 63-64) listed *Mustela c. cicognani* (short-tailed weasel) and *M. frenata* (long-tailed weasel) as host of *Physaloptera* sp. Species identification was not made due to lack of mature male or female specimens, but they were probably *P. maxillaris*. This material was kindly sent to the writer by Dr. A. B. Erickson, University of Minnesota.

Molineus patens (Dujardin, 1845) was found in the small intestine of *Mustela r. rixosa* (least weasel) and *M. v. mink* (common mink). The least weasel appears to be a new host for this trichostrongylid nematode. North American records of this parasite are infrequent. Skinker (1932, J. Parasitol. 19: 94) reported *M. patens* for the first North American records in a "mink" from Mississippi and *Canis lestin* (Great Basin coyote) from Washington. European hosts of *M. patens* include *Putorius putorius* (ilts) Germany, *Mustela nivalis* (weasel) England, *M. erminea* (ermine or stoat) England, *M. sibiricus* (Siberian weasel) Russia, *Vormela sarmatica* (marbled polecat) and *Martes foina* (beech marten), all members of the Mustelidae.

Some nematodes forwarded to the Zoological Division, Washington, D. C., from the stomachs of 2 martens, *Martes a. americana* (American marten) and *M. p. pennanti* (fisher) were identified by Dr. E. W. Price as *Soboliphyme baturini* Petrow, 1930. Price (1930, J. Parasitol. 17: 57) reported the first North American record from Montana of this parasite in the stomach of *Gulo* sp. (wolverine), Mustelidae. The above hosts are recorded as new. Hosts reported by Petrow (1930, Zool. Anz. 86: 265-272) from Russia were *Martes sibirica* (marten), *Vulpes vulpes* (fox), and *Felis domestica* (domestic cat).

Ascaris sp. probably *A. columnaris* Leidy, 1886, described from skunks and raccoons were recovered from the small intestine of 2 badgers (*Taxidea t. taxus*). One badger harbored a large number of *Physaloptera torquata* Leidy, 1886. Other North American hosts for this parasite are *Taxidea t. neglecta* (western badger) and *Procyon l. lotor* (eastern raccoon).—BANNER BILL MORGAN, Department of Veterinary Science, University of Wisconsin.

ANIMAL PARASITES OF THE FOX SQUIRREL, *SCIURUS NIGER RUFIVENTER*, IN SOUTHEASTERN KANSAS

Approximately thirty species of animal parasites have been reported from the fox squirrel, *Sciurus niger rufiventer*, from widely scattered areas but mostly from the central states. Apparently very few specimens of this host have been examined systematically for all kinds of animal parasites.

A considerable number of reports on parasites of the fox squirrel has been published, but in most instances only one or two species of parasites was studied. Most of this literature is listed by Harkema (1936, Ecol. Monogr. 6: 151-232) and by Katz (1939, Ohio Wildlife Res. Sta., Release No. 131). Two relatively recent and notable exceptions are Katz's report of eleven species of parasites from sixteen fox squirrels in Ohio and Chandler's (1942, J. Parasitol. 28: 135-140) report of six species of helminths from thirteen fox squirrels in southeast Texas.

One hundred fox squirrels, *Sciurus niger rufiventer*, from southeastern Kansas during the period of October, 1939, to July, 1940, were examined for all animal parasites. The chief results of this investigation are summarized in Table 1.

Of the sixteen parasites listed in the table the following have been previously reported from the fox squirrel:

Eimeria sp., *Taenia pisiformis*; *Heligmodendrium hassalli*, *Rictularia* sp., *Orchopeas wickhami*, *Neohaematopinus sciurinus*, *Dermacentor variabilis*, Sarcoptidae, and *Trombicula* sp.

Pathological conditions were observed only in the squirrel infected with the itch mite. Emaciation, deformed vertebrae, and loss of hair were observed in this animal. The maximum number of species of parasites found in any host was six. No parasites were found in seven squirrels.

TABLE 1.—Parasites found in 100 squirrels (*Sciurus niger rufiventer*) examined from southeastern Kansas, 1939–40

Name of parasite	Squirrels infected	Parasites per infected squirrel	
		Average	Maximum
<i>Eimeria</i> sp.	65	Many	Very many
Amoeba (8-nucleate cyst)	1	Very many	Very many
Coccidia	1	Few	Few
<i>Catenataenia</i> sp.	2	1	1
<i>Taenia pisiformis</i> (larva)	1	4	4
<i>Heligmodendrium hassalli</i>	17	12	72
<i>Trichostrongylus calcaratus</i>	7	7	11
<i>Rictularia</i> sp.	1	2	2
<i>Ascaris</i> sp. (immature)	1	1	1
<i>Hoplosyllus affinis</i> and <i>Orchopeas wickhami</i>	63	6	40
<i>Neohaematopinus sciurinus</i>	46	15	115
<i>Dermacentor variabilis</i>	7	2	5
Sarcoptidae	1	Very many	Very many
<i>Trombicula</i> sp.	15	3	21
<i>Atricholaelaps glasgowi</i>	1	3	3

It may be of interest to note that of the species of parasites listed in Table 1, only four are found in the list of eleven species reported by Katz. Furthermore, of the seven species of helminths listed in Table 1, the six reported by Katz, and the six reported by Chandler, only one species was included in more than one of these reports. *Heligmodendrium hassalli* is common to all three studies.

Dr. E. R. Becker, Dr. H. E. Ewing, Dr. E. W. Price, Mr. J. T. Lucker and Mr. Allen McIntosh kindly aided in the identification of the parasites.—EDWARD GRAHAM AND JACOB UHRICH, *Kansas State Teachers College, Pittsburg, Kansas.*

STRONGYLOIDES PLANICEPS, NEW NAME FOR *S. CATI* ROGERS

The name *Strongyloides planiceps* is advanced to replace the homonym *Strongyloides cati* Rogers (1939, J. Helm. 17: 229–238) in connection with a parasite found in the domestic cat but originally obtained from *Felis planiceps*. The first use of the name *S. cati* appears to be by Brumpt (1927, Précis de Parasitologie, Masson et Cie, Paris) in a footnote on page 662, "Au Bengale, le Chat présente, dans 20 pour 100 des cas sur 250 examinés, un *Strongyloides* identifié au *stercoralis* par A. Chandler (1925) et considéré comme une espèce particulière, *S. cati*, par d'autres auteurs." Attempts to locate the "autres auteurs" have failed and it appears possible that the name *S. cati*, unaccompanied by an indication as it was first used, is a nomen nudum.

The help given by Dr. Benjamin Schwartz who pointed out that the name *S. cati* was a homonym and who has endeavored to trace the authors referred to by Brumpt, is gratefully acknowledged.—WILLIAM P. ROGERS, *Institute of Agricultural Parasitology, St. Albans, England.*

SPECIMENS NEEDED BY THE MEDICAL SCHOOLS

The Department of Parasitology and Tropical Medicine, Army Medical School, Washington, D. C., is serving as a Distributing Center for Parasitological Specimens under the auspices of a committee formed by the American Association of Medical Colleges and the National Research Council. The Department is functioning as a collecting and distributing agency for gross and microscopic specimens of protozoa, parasitic worms and ova, arthropods of medical importance, malaria and other blood parasites. If you or your institution possess any specimens which would be valuable to medical schools for these purposes and which you are willing to contribute, please send them to the Director, Army Medical School, Army Medical Center, Washington, D. C. Mailing containers and shipping directions will be furnished upon request. Your cooperation in these matters will be greatly appreciated.

GEORGE W. HUNTER, III,
Major, Sanitary Corps.

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HELMINTHS FROM THE ROBIN, WITH THE DESCRIPTION OF A NEW NEMATODE, *PORROCAECUM BREVISPICULUM*

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In the autumn of 1939 the writer examined thirteen robins (*Turdus migratorius*) taken near Ithaca, New York, mostly by Dr. Joseph C. Howell, who also contributed parasites and data from several other robins taken in the same locality in 1937. The following worms were found: one trematode, *Lyperosomum monenteron* Price and McIntosh, 1935 (DICROCOELIIDAE); two cestodes, *Hymenolepis planestici* (Mayhew, 1925) Fuhrmann, 1932 (HYMENOLEPIDIDAE) and *Dilepis undula* (Schränk, 1788) Fuhrmann, 1908 (= *Southwellia ransomi* Chapin, 1926 according to Fuhrmann [1932:100]) (DILEPIDIDAE); four nematodes, *Porrocaecum ensicaudatum* (Zeder, 1800) Baylis, 1920; *Porrocaecum brevispiculum* n. sp. (ASCARIDIDAE); *Acuaria (Dispharynx) spiralis* (Molin, 1858) Railliet, Henry, and Sissof, 1912 (SPIRURIDAE); and *Syngamus trachea* (Montagu, 1811) Chapin, 1925 (STRONGYLIDAE); one acanthocephalan, *Plagiorhynchus formosus* Van Cleave, 1918 (ACANTHOCEPHALIDAE).

Lyperosomum monenteron, which has previously been reported from the bluebird in Virginia and the robin in Quebec, Virginia, and Washington, D. C., by Price and McIntosh (1935), and from the robin and mockingbird in Texas by Denton (1942), was found in three of thirteen robins in which the liver and bile ducts were examined. *Hymenolepis planestici* was found in four of nineteen birds and *Dilepis undula* in nine of nineteen.

Thirty-odd specimens of the genus *Porrocaecum* were taken from the intestines of seven of 19 robins examined, but specific identification was not made until after record was lost of the particular host of each individual worm. Five of these specimens—four males and one female—are assigned to *P. ensicaudatum*; the rest belong to a new species. These five individuals all lack lateral alae and otherwise agree with Baylis' (1922) diagnosis of *P. ensicaudatum*, save that two males had very long spicules that necessitate using Lewis' (1926) emendation—i.e., a greater maximum spicule length than the 620 to 630 μ designated by Baylis. Lewis' other emendation—occasional presence of lateral alae in *P. ensicaudatum*—is evidently applicable to North American material, because at least one of the five specimens from the Leidy Collection identified by Walton (1927:63) as of this species posses-

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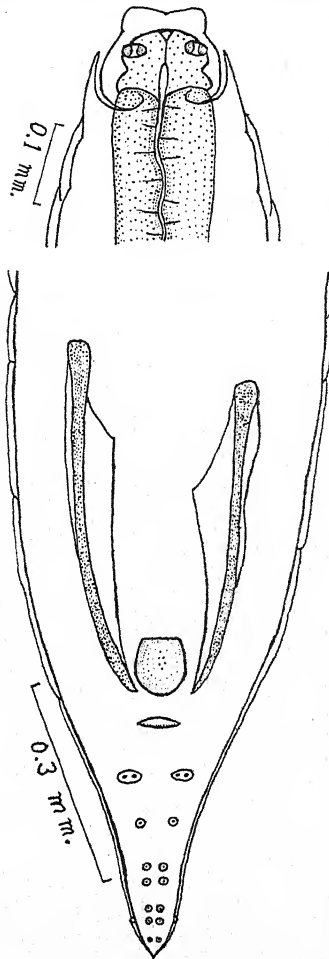
*The writer wishes to extend his thanks to Dr. Joseph C. Howell for robins and parasite data, to Dr. D. H. Wenrich and the University of Pennsylvania for the loan of the Leidy specimens, to Dr. H. J. Van Cleave for the identification of *Plagiorhynchus formosus*, and to Drs. Asa C. Chandler and Donald W. Baker for their help and advice.

ses lateral alae. The writer concurs in this identification. The Leidy specimens were from the mockingbird, but *P. ensicaudatum* has previously been listed from the robin by Cram (1932) and Rayner (1932).

Porrocaecum brevispiculum n. sp.

(Figs. 1 and 2)

Diagnosis. *Porrocaecum*: Lateral alae present, little wider on neck than anywhere else. Each lip with a deep groove on either side of base, and a dentigerous ridge on anterior end. All



Porrocaecum brevispiculum n. sp.

FIG. 1 (upper). Dorsal aspect of lips of male. FIG. 2 (lower). Tail of male showing alate spicules, gubernaculum, and postanal papillae.

twelve external cephalic papillae well developed; incompletely fused in pairs. Three slender interlabia extend to just half length of ventriculus.

Males. Seven specimens measured 18 to 30 (av. 23.8) mm long and 526 to 794 (av. 611) μ wide. Dorsal lip 179 to 198 (av. 193) μ wide. Esophagus 1.53 to 2.10 (av. 1.69) mm long or 6 to 7% of body length. Ventriculus 313 to 632 (av. 398) μ long; cecum 22 to 124 (av. 88) μ long. Seven or eight pairs of postanal papillae present; one pair, doubles, just posterior to anus, one pair laterally very near tail, five or six pairs ventrally. Ventral pair of papillae immediately anterior to lateral pair sometimes absent. A barrel-shaped gubernaculum present, 85 to 190 μ long, placed between spicules, immediately anterior to anus, and partially surrounded by spicular

alae. Alate spicules subequal, left longer, 346 to 567 (av. 470) μ and right shorter, 256 to 487 (av. 408) μ . Tail, measured from posterior border of anus to tip, 208 to 471 (av. 295) μ long.

Females. Six specimens measured 28 to 42 (av. 36) mm long and at vulva 612 to 727 (av. 671) μ wide. Dorsal lip 225 to 249 (av. 233) μ wide. Esophagus 1.55 to 2.66 (av. 2.01) mm long or 5 to 7% of body length. Ventriculus 403 to 502 (av. 417) μ long. In one case cecum slightly exceeded half length of ventriculus. Tail 583 to 809 (av. 659) μ long, relatively thick and blunt as compared with male tail. Vulva somewhat posterior to junction of first and second thirds of body, dividing it in ratio of from 1/2.1 to 1/2.6. Eggs ellipsoid; outer shell with cobble-stone-like surface paved with irregularly rounded plates; eggs 81.1 to 96.5 (av. 91.3) μ by 56.0 to 64.3 (av. 60.9) μ .

Host: *Turdus migratorius*.

Habitat: Small intestine.

Locality: Ithaca, New York.

Type: Male, U. S. N. M. Helm. Coll. No. 40687; paratype—female, U. S. N. M. Helm. Coll. No. 40687.

Porrocaecum brevispiculum is distinguishable from the closely related *P. cheni* Hsü, 1933, by the presence in the former of lateral alae, a gubernaculum, shorter spicules, and a more anteriorly-placed vulva. The other species with a rudimentary intestinal cecum, *P. ensicaudatum*, lacks a gubernaculum, and possesses lateral wings on the pulp of each lip and longer spicules than are found in *P. brevispiculum*. Possibly the immature *Porrocaecum* which Chapin (1926) mentions taking from a Washington, D. C., robin were also of this species.

Acuaria spiralis was found in the stomachs of five of 19 robins; in one case about 150 worms had eroded most of the proventricular mucosa. A single *Syngamus trachea* was found in the nineteen robins examined.

Dr. Howell found three robins, each of which was dying, apparently from the pathogenic effects of one or two acanthocephalans, identified as *Plagiorhynchus formosus*. Pathogenic effects of this parasite upon the robin have previously been reported by Jones (1928).

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THE DEVELOPMENT OF THE SPOROCASTS OF A SCHISTOSOME, *CERCARIA STAGNICOLAE* TALBOT, 1936¹

W. W. CORT AND LOUIS OLIVIER²

During the summers of 1940 and 1941 at the University of Michigan Biological Station we made a study of the mother and daughter sporocysts of *Cercaria stagnicola* Talbot, 1936, from natural infections in *Stagnicola emarginata angulata* (Sowerby). *C. stagnicola* is the commonest schistosome in the Douglas Lake Region and is the most important cause of swimmers' itch in Michigan (McMullen and Brackett, 1941). Large numbers of infections of this cercaria come to maturity in adult snails in late June and early July; on the other hand, infections are very rare in juvenile snails from the time they begin to hatch in early June through the first third of September (Cort et al, 1937; Cort et al, 1940). Therefore, it was concluded that the snails acquire most of their infections with *C. stagnicola* late in the fall and early in the spring. Thus, the examination of adult snails in May and June would offer the best opportunity to study the early development of the sporocysts of this species. Our studies were actually made from June 26 to July 11, 1940, and from June 20 to July 3, 1941. Even this late we found numerous mother sporocysts, some still immature, and followed the early development of the daughter sporocysts; but as was to be expected very early stages of mother sporocysts were not found.

The methods used in this study were the same as those employed in our earlier investigations (Cort and Olivier, 1941). *C. stagnicola* is the only schistosome that has ever been found in *S. e. angulata* in the region with the exception of a single infection of *Schistosomatium douthitti* (Cort et al, 1937). Schistosome mother and daughter sporocysts are easy to differentiate from those of strigeids and plagiogochiids; and after we had once determined the position of the mother sporocysts in the snails they were not difficult to find. In all, we obtained more than fifty mother sporocysts of this species and were able to make more or less detailed observations on more than thirty of them. About half of these were considered to be mature since they contained daughter sporocysts that appeared ready to emerge. We were also able to obtain for study a number of immature daughter sporocysts.

MOTHER SPOROCASTS OF *C. stagnicola*

The mother sporocysts of *C. stagnicola* are very thin walled, sausage shaped sacs, which, even when they contain numerous well developed daughter sporocyst embryos, appear quite empty, with the germinal material filling only a fraction of the inflated lumen. The youngest that were found were about 3 mm in length and from 0.10 to 0.17 mm in width, and the largest were over 10 mm long and about 0.2

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This is one of a series of investigations on the early developmental stages of digenetic trematodes in the snail hosts. Previous studies have been on strigeids (Cort and Olivier, 1941) and a plagiogochiid (Cort and Olivier, 1943).

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to 0.3 mm broad. These measurements of width are probably too small because freed sporocysts whether broken or not usually became more or less deflated.

Most of the mother sporocysts were located on the surface of the kidney and stomach of the snails; others were found on the surface of the uterus and nidamental gland, along the intestine, and in the proximal part of the digestive gland. In all cases they were more or less firmly laced for a part of their length into the connective tissue covering the organs; and when the snails were removed from their shells for examination portions of the mother sporocysts could frequently be seen sticking out from the surfaces of the organs. Both when in position in the tissues of the host and when placed in the saline solution for study their power of movement was very limited, their activity being very much less than that of the thicker walled mother sporocysts of strigeids. One end is more attenuated than the other and except in very immature specimens a birth pore and canal can be clearly seen (Fig. 1).

The body wall of the mother sporocysts of *C. stagnicolae* has a very thin cuticula and a layer containing flattened stellate cells with rather small nuclei. Circular and longitudinal muscle elements must be present but could not be seen in living material. The cellular layer is thicker and its nuclei are more numerous in a given area in the younger as compared with the older sporocysts. The stellate shape of the cells of this layer can be seen best in surface view as shown in Fig. 2. In the younger mother sporocysts the wall is colorless and translucent, but as they grow older it becomes more and more greyish in appearance from the accumulation in the cellular layer of minute, oily droplets. Few flame cells were ever seen in the wall, whereas in strigeid mother sporocysts flame cells are numerous and very conspicuous. In the youngest mother sporocysts of *C. stagnicolae* the body cavity was traversed by numerous protoplasmic processes; but in the older ones these processes were absent and the contents moved back and forth freely.

In the smallest mother sporocysts most of the germinal material was attached to the protoplasmic processes which extended from the body wall. It consisted of single germ cells, groups of a few germ cells, germ balls which appeared to be very young daughter sporocyst embryos, and germ masses with both unicellular and multicellular components. There were comparatively few isolated germ cells, due probably to the fact that our earliest stages were about 3 mm in length. In one of the youngest mother sporocysts studied, however, there were regions where only single germ cells were present attached to the processes that traversed the cavity (Fig. 3). Fig. 4 shows a part of another very immature sporocyst showing single germ cells, irregular groups of three to several germ cells, and some germ balls. In two other very immature mothers there were groups composed of small germ balls and germ cells, which were held in the lumen of the cavity by the processes from the wall. These groups of germinal material, which may be considered to be "germ masses," were separated by empty spaces not traversed by the processes from the wall. They were held firmly in position and did not move back and forth in the lumen. Fig. 5 shows the posterior end of one of these two sporocysts in which the position and structure of five such "germ masses" are shown.

In what appeared to be a slightly older mother sporocyst there was present a number of oval daughter sporocyst embryos floating free in the cavity with no strands connecting them with the wall. In a few places in this sporocyst, however, strands could be seen with single germ cells and small groups of germ cells attached to them (Fig. 6).

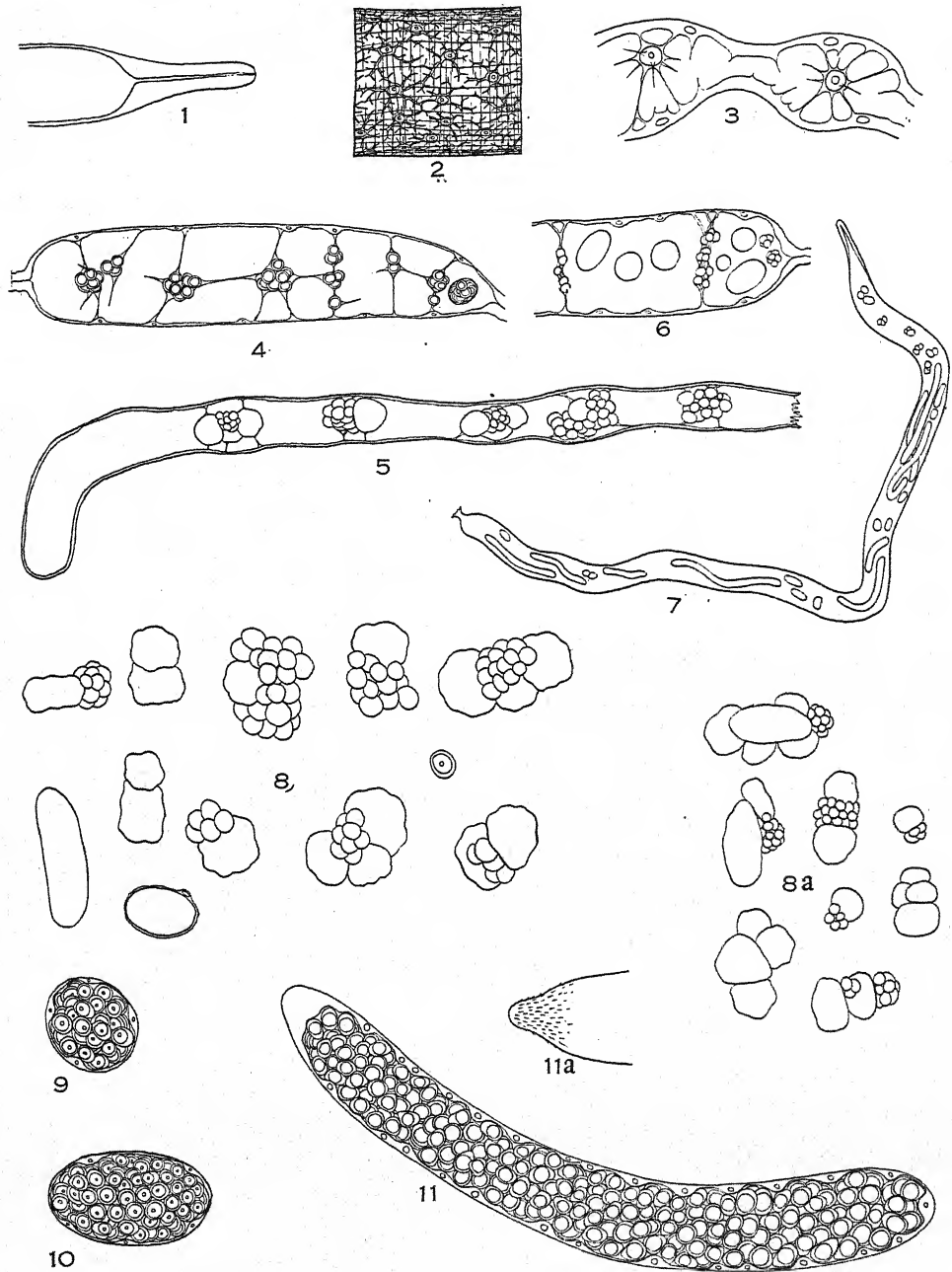


FIG. 1. Anterior end of immature mother sporocyst showing birth pore and canal.

FIG. 2. Surface view of wall of mother sporocyst showing the characteristics of the cells.

FIG. 3. A small section of a very immature mother sporocyst showing germ cells suspended by strands from the wall.

FIG. 4. Section of immature mother sporocyst showing single germ cells and cell groups suspended by strands from the wall.

FIG. 5. Posterior end of immature mother sporocyst showing 5 germ masses attached by strands to the wall.

A somewhat later stage was represented by another immature mother sporocyst that was freed unbroken and mounted whole for study. Two of its daughter sporocyst embryos had become somewhat elongate; the others were in earlier developmental stages. Its anterior end was attenuated and the birth pore and canal could be made out. Close to this end there was a group of a few germ cells held by strands from the wall. A little further along there was an irregular germ mass and beyond this several small germ balls (daughter sporocyst embryos), also, attached to the wall. Throughout the rest of the length of this sporocyst no further processes crossing the lumen were present and the contents which consisted of numerous daughter sporocyst embryos floated freely back and forth. In all over 60 embryos were counted in this mother.

Mother sporocysts were considered to have reached maturity when they contained fully developed daughter sporocyst embryos that moved actively and appeared ready to escape (Fig. 7). In some infections with mature mother sporocysts, immature daughters that had already escaped into the snail tissues were present. There were even a few cases in which old mother sporocysts still containing a few embryos persisted when some of their daughter sporocyst brood had become mature and contained fully developed cercariae. As already noted the mature mother sporocysts were larger and thinner walled than the immature ones and appeared greyish. The sporocyst cavity was not traversed by strands and its contents, which only filled a fraction of the lumen, moved freely back and forth. All stages of daughter sporocyst embryos were present in mature mother sporocysts including elongate active forms that appeared ready to escape. In addition, there were also present a few irregular masses of germinal material, some of which included both germ balls and single cells, and others only germ balls. These germ masses were irregular in shape, varied greatly in size and composition, and did not seem to have a membrane of their own. Figs. 8 and 8a show a series of them from several different mature mother sporocysts. These free floating germ masses of the mature mother sporocysts, which, as noted above, are also found in immature mothers, are like the masses found attached to the protoplasmic strands in some of the younger sporocysts (Fig. 5). They probably are masses of the same type which have been freed from the strands connecting them with the wall. Usually, also, a few isolated germ cells were found free in the lumen of mature mother sporocysts. These may have broken loose from floating germ masses.

In a few cases it was possible to make counts of the germinal elements present in mature mother sporocysts. One complete, large, well developed mother sporocyst contained 6 motile, elongate daughter sporocysts which appeared about ready to escape, 13 elongate embryos, 11 that were large and oval, and over 40 earlier

FIG. 6. Section of immature mother sporocyst showing germinal material on strands from body wall and free germ balls (daughter sporocyst embryos).

FIG. 7. Anterior portion of mature mother sporocyst, showing daughter sporocyst embryos in different stages of development and germ masses.

FIG. 8. Series of germ masses and two daughter sporocyst embryos for size comparison.

FIG. 8a. Germ masses from mother sporocysts drawn to smaller scale.

FIG. 9. Daughter sporocyst embryo in germ ball stage. Diameter 0.049 mm.

FIG. 10. Daughter sporocyst embryo beginning to elongate. Length, 0.094 mm.

FIG. 11. Daughter sporocyst ready to escape from mother (migrating stage). Length, 0.371 mm; width, 0.051 mm. Specimens from which this and two previous drawings were drawn were somewhat flattened.

FIG. 11a. Anterior tip of daughter sporocyst showing spines.

embryos in the germ ball stage. There were, also, a few germ masses, some of which contained both multicellular and unicellular components. No separate germ cells were seen in this particular sporocyst. It appeared to have just reached maturity since a careful search revealed no developing daughter sporocysts in the snail's tissues. In another mature mother sporocyst there were over 100 daughter sporocyst embryos in all stages of development and six germ masses. Another contained over 25 elongate, motile daughter sporocysts and a number of younger embryos at various stages. A part (length 4.2 mm) of an old mother sporocyst had 9 elongate, motile daughter sporocysts, 15 younger developing embryos, and a single germ mass consisting of one germ ball to which several single cells were attached. In this infection developing daughter sporocysts, the oldest of which was almost mature, were found in the digestive gland of the snail. In another old mother sporocyst, some of whose daughters had emerged and reached maturity, there were still 5 fully developed daughter sporocyst embryos, 28 others in all stages of development, and 8 "germ masses" of which half still showed single germ cells. In most of the old mother sporocysts, however, the amount of embryonic material was much less.

STAGES OF DEVELOPMENT OF DAUGHTER SPOROCYST EMBRYOS

The smallest daughter sporocyst embryos within the mothers were small, spherical germ balls which were just like the larger multicellular components of the germ masses. In some of the larger of these germ balls it was possible to see a thin, outer membrane; and in some, about 0.02 to 0.03 mm in diameter, differentiated, flattened cells of the enclosing membrane could occasionally be made out. When embryos 0.04 to 0.05 mm in diameter were stained lightly with neutral red, it was possible to see clearly the relation of the wall and contents (Fig. 9). Such embryos consisted of a group of large distinctly separate germ cells, enclosed by a thin wall composed of flattened cells, with small nuclei and coarsely granular, vacuolated cytoplasm. The germ cells had large, clear nuclei, conspicuous nucleoli, and a thin, outer layer of homogeneous, finely granular cytoplasm. They were like the germ cells found free or in germ masses in the mother sporocysts. Somewhat larger embryos, 0.09 to 0.10 mm in length and 0.06 to 0.07 mm in width, had the same structure except that the germ cells were more numerous (Fig. 10). The structure was, also, essentially the same in older, elongate daughter sporocyst embryos, which were from 0.25 to 0.30 mm in length and 0.03 to 0.04 mm in width. In them the body wall was thicker and much more differentiated and the number of germ cells in the cavity was considerably larger.

The motile daughter sporocysts (migrating stage) which appeared ready to escape from the mother were studied carefully using neutral red as an *intra vitam* stain (Fig. 11). They moved actively and extended and contracted the body considerably. A series of ten from one mother sporocyst, measured alive had an average size of 0.37 by 0.05 mm, and the largest seen was 0.64 mm in length. Their anterior end is mobile and tapering and bears numerous small spines (Fig. 11a). They have a thin cuticula and well developed muscle layers. No spines except those on the anterior end were present and no cilia could be seen on their surface, even when they were studied with dark field illumination at magnifications up to 1000 times. In this respect they differ from the description given by Price (1931) for the immature daughter sporocysts of *S. douthitti*. The body cavity of these daughter

sporocysts is crowded with large numbers of germ cells (over 100 counted in one case) which are not attached to each other or to the body wall. These germ cells are tightly packed into the cavity and completely fill it for its whole length; no multicellular groups were ever seen at this stage. Each daughter sporocyst has two terminal, posterior excretory pores. It seems probable that normally the daughter sporocysts escape only by way of the birth pore of the mother, and in one case one was seen in the act of escaping (Fig. 12).

DEVELOPMENT OF THE DAUGHTER SPOROCASTS OUTSIDE THE MOTHER

After leaving the mother, most of the daughter sporocysts migrate into the digestive gland of the snail, although they may become located on the surface of the organs in front of this gland. They penetrate into all parts of the parenchyma of the digestive gland and develop in intimate contact with the tissues. When found among the organs in front of the digestive gland they are also in the connective tissues. Since the daughter sporocysts are so thin walled and are in such intimate contact with the snail's tissues, it proved very difficult to free them unbroken, especially after they had increased considerably in size.

The daughter sporocysts that have just escaped from the mother sporocyst have the same structure as that described above for those still in the mother. Their great mobility and probably the spines at the anterior end make migration possible. Since these migrating daughter sporocysts grow directly into the large mature daughter sporocysts it is obvious that they increase greatly in size. Also, there must be some method of multiplication of their germ cells, since, from what we know of cercarial production of this schistosome infection, it seems clear that there are not enough germ cells present in a migrating daughter, if each developed into one cercaria, to account for the large numbers of cercariae produced.

Some very early stages in the development of the free daughter sporocysts were available for study. One of the youngest found, which was about 0.5 mm in length is shown in Fig. 13. It can be seen that for about the anterior one-third this sporocyst contained no germinal material, and showed strands of tissue traversing the body cavity. The middle third was only partly filled with germinal material, most of which consisted of single cells with only a few multicellular groups. The posterior third was crowded with separate germ cells.

Another immature daughter sporocyst of about the same stage of development is shown in Fig. 14. Its body cavity contained large numbers of single germ cells, but there were about 14 multicellular groups. Some of these groups were made up of only two to four cells, but several had considerably larger numbers. Each group evidently came from the division of one of the single germ cells, but it could not be determined whether they were developing directly into cercarial embryos or would become germ masses. The whole body cavity of this sporocyst was completely filled with germinal material except the posterior end and there were no protoplasmic processes traversing the lumen.

Only the posterior end of another very young daughter sporocyst was obtained for study (Fig. 15). In this specimen the body cavity was traversed by protoplasmic strands on which were held both single germ cells and multicellular groups.

A portion of another very immature daughter sporocyst was also studied (Fig. 16). In it the germinal material only filled a fraction of the body cavity, which was traversed by numerous protoplasmic strands. Held on these strands were numerous

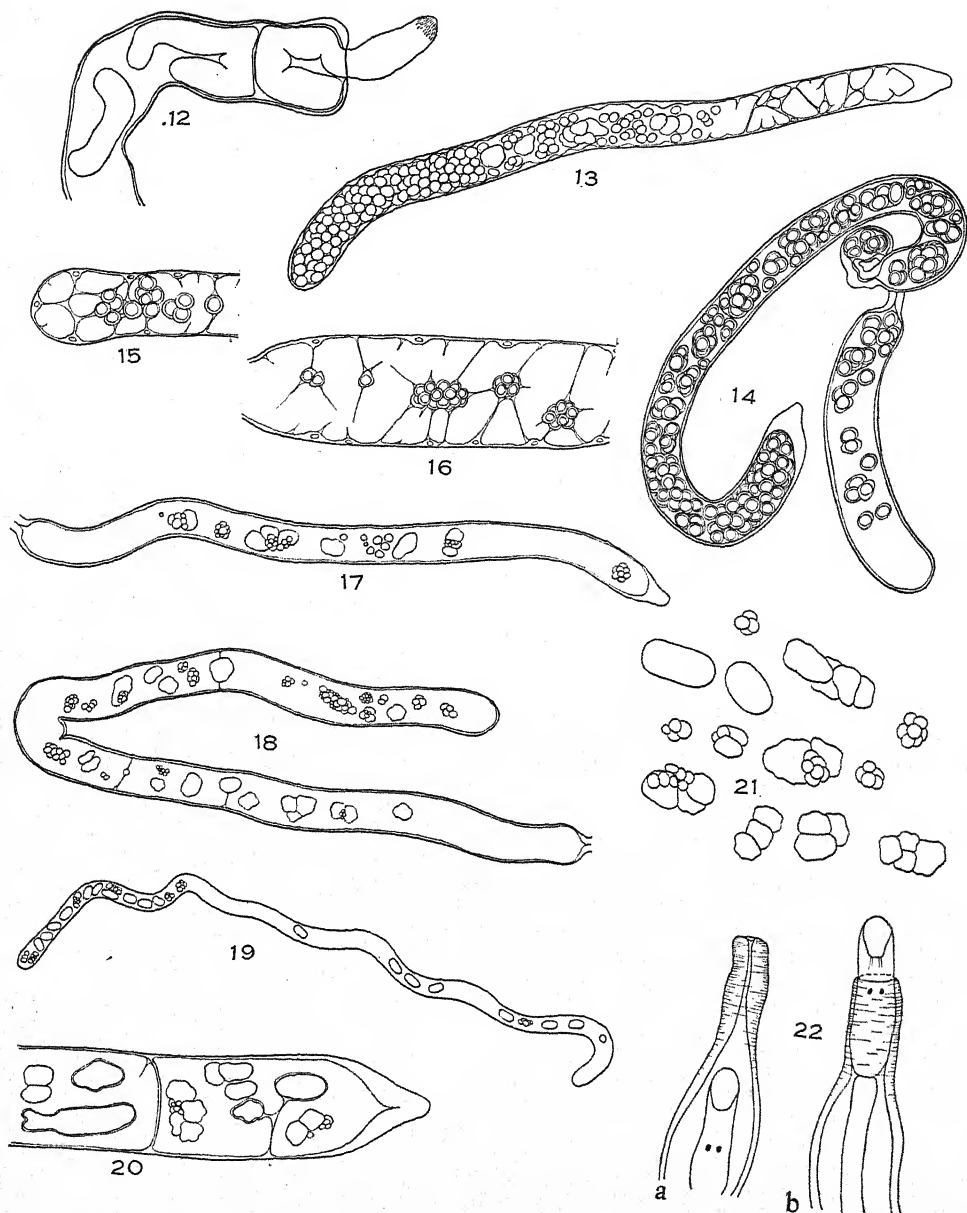


FIG. 12. Daughter sporocyst escaping through birth pore of mother. Specimen much flattened.

FIG. 13. Early stage of daughter sporocyst soon after escape from mother. Length, 0.65 mm.

FIG. 14. Very immature daughter sporocyst after escape from mother sporocyst.

FIGS. 15 and 16. Parts of immature daughter sporocyst showing attachment of germinal material to wall by strands.

FIGS. 17 and 18. Pieces of immature daughter sporocysts with germinal material floating free in body cavity.

FIG. 19. Immature daughter sporocyst in which cercarial embryos are beginning to show differentiation.

FIG. 20. Anterior end of slightly older daughter sporocyst in which oldest embryo shows differentiation into body and tail.

FIG. 21. Germ masses from daughter sporocysts. Two daughter sporocyst embryos included for size comparison.

FIG. 22 a and b. Anterior ends of mature daughter sporocysts showing birth pore and canal.

separate germ cells, some early cleavage stages of two to four cells, and a few groups of up to 16 cells. The largest cell groups did not look like cercarial embryos since the cells in them were only loosely held together.

Several other very immature daughter sporocysts in which the body cavity was traversed by protoplasmic strands bearing the germinal material were also observed. In all these sporocysts most of the lumen was empty. Such sporocysts usually contained some irregular germ masses. Immature sporocysts were also found in which there were very few protoplasmic strands traversing the body cavity and which had free in the lumen germinal material consisting of single germ cells, groups of a few cells, and irregular germ masses composed of both unicellular and multicellular components (Figs. 17 and 18).

Daughter sporocysts that appeared to be in later stages of development contained numbers of structures that could be definitely recognized as cercarial embryos. One such sporocyst which was removed unbroken is shown in Fig. 19. It measured 4.5 mm in length and 0.13 mm in width. No protoplasmic strands could be seen crossing the body cavity. It contained a surprisingly small amount of germinal material, since its total contents consisted of 7 irregular germ masses 0.040 to 0.050 mm in diameter and 18 cercarial embryos 0.050 to 0.075 mm in length, none of which showed differentiation into body and tail. Another immature daughter sporocyst that was freed intact contained no single germ cells, about 25 to 30 germ masses and a few small cercarial embryos. The small amount of germinal material in these two sporocysts was rather surprising and appeared to be much less than in most of the immature daughter sporocysts seen.

A number of daughter sporocysts were observed at a somewhat older stage when the oldest cercarial embryos were just beginning to show differentiation into body and tail. These, also, contained numerous cercarial embryos in the germ ball stage and a varying number of germ masses. In only a few cases were there any protoplasmic strands in the body cavity and no separate germ cells were seen. Fig. 20 shows the anterior end of a sporocyst in this stage which still retained a few protoplasmic strands.

A number of daughter sporocysts approaching maturity were also studied. In general, most of their contents consisted of immature cercariae at approximately the same stage of development, which filled most of the cavity. In most of these sporocysts small numbers of young embryos and a few germ masses were also present. Even in sporocysts that contained mature cercariae germ masses and very young embryos were sometimes found. Frequently, however, only fairly well developed cercariae were present in mature sporocysts.

The germ masses of the daughter sporocysts of *C. stagnicolae* are similar to those of the mother. A series of them from several different sporocysts of different ages is shown in Fig. 21. Many of them have only multicellular components, usually varying in number from two to five; but others are made up of both multicellular and unicellular components. They vary greatly in shape, size, and number of components, and do not seem to have a membrane surrounding them. The largest multicellular components of the germ masses correspond in structure to the smallest cercarial embryos (Fig. 21).

Mature daughter sporocysts have a thin, fragile wall which contains varying amounts of minute, refractile, oily droplets giving them a more or less opaque ap-

pearance. It appears that the older the sporocysts are, the more numerous are the globules in the wall so that in old infections the sporocysts appear quite black in transmitted light. Mature sporocysts are almost completely non-motile, except as influenced by the movements of the cercariae that they contain and are tightly laced into the tissue of the snail. It was very difficult to free them for study; in fact, in no cases were we able to isolate a complete one. In some infections producing mature cercariae some of the sporocysts contained only immature cercariae. Often when all the sporocysts had reached maturity there would also be present varying numbers of partially or entirely empty sporocysts which had collapsed and were degenerating.

Mature daughter sporocysts have an attenuated anterior end with a birth pore and canal, which are very conspicuous when cercariae are emerging (Figs. 22 a and b). The best way to see the birth pore is to pull the body of a snail containing a mature infection out of the shell, and observe the surface of the digestive gland in water or saline solution. In such preparations the anterior ends of numerous sporocysts may be seen projecting out from the surface of the digestive gland. In a short time cercariae will begin to emerge from the birth pores at the free ends. Several cercariae have been observed to emerge in a few minutes from the free end of a single sporocyst.

DISCUSSION

Although our picture is not complete, we can give a general outline of the probable development of the sporocysts and the course of the germ cell cycle of *C. stagnicola*. Postulating that the miracidium of this species is like those of other schistosomes, it can be suggested that its body cavity contains a considerable number of germ cells attached to the wall by protoplasmic processes (Price, 1931, Pl. 2, Fig. 13; Tang, 1938, Pl. II; Wall, 1941, Fig. 19). In the early stages of the metamorphosis of the miracidium into the mother sporocyst a very rapid and extensive growth of the body wall takes place to produce the elongate sacs, which are the earliest mother sporocysts which we found. In these, germinal development is far outstripped by somatic growth since the germinal material only fills a small fraction of the body cavity. Since we found in our youngest stages separate germ cells in the cavity suspended by strands from the body wall, it appears that as the miracidium metamorphoses into an inflated elongate sac, its germ cells become distributed throughout the cavity by the extension of the strands which connect them with the body wall. Some of these germ cells may possibly develop directly into daughter sporocyst embryos without any multiplication, and others may divide into a few germ cells which later separate to form embryos. However, the finding of irregular, rather loosely organized germ masses, which in the younger stages are attached to the body wall by strands and which in later stages float free in the cavity, suggests that a phase of multiplication by polyembryony is commonly present. It seems probable that each of these germ masses is the product of a single germ cell. If, when a germ cell divided, the daughter cells remained loosely attached to each other and later one by one started to develop into embryos, the result would be the production of germ masses containing both multicellular and unicellular components like those shown in Figs. 8 and 8a. Then, after all the germ cells of a germ mass had started to develop into embryos, it would come to be composed only of multicellular components, which would break away one at a time until the mass was entirely disintegrated. The terminal stages would, therefore, be germ masses containing several or finally only two multicellular

components (Fig. 8). As more and more embryos became free in the cavity and as some of them developed into elongate, active daughter sporocysts, their movements, both passive and active, would tend to break down the protoplasmic strands across the lumen, and soon all the germinal material would float free in the cavity. Thus, germ masses would be found free in the cavity along with embryos in various stages of development.

There must be a considerable lag in the development of some of the germ masses of the mother sporocysts since a few are still present in mature and even old sporocysts. This lag would make possible the production of new daughter sporocysts throughout the whole reproductive life of the mother. In fact, a few very immature embryos were still present in old mother sporocysts even when the first daughters that had escaped already contained fully developed cercariae. However, the life of the mother sporocysts in *C. stagnicolae* appears to be relatively much shorter than in the strigeids since it is rare to find mother sporocysts in mature infections.

In the development of the daughter sporocyst embryos inside the mother there is a rapid multiplication of germ cells. In very early stages (germ balls) a few cells are differentiated which form the wall of the sporocyst and all the others go on multiplying until at the time of emergence from the mother the daughter sporocyst contains over a hundred separate germ cells which completely fill the body cavity (note stages of development in Figs. 9, 10, and 11). In spite of the large number of separate germ cells in the migrating stage of the daughter sporocysts of *C. stagnicolae* there must be further multiplication of germinal material to produce the large numbers of cercariae that develop. Consequently, as in the mother sporocyst, a phase of multiplication by polyembryony is introduced. After emergence from the mother the somatic development of the daughter sporocyst outstrips the germinal, since a stage is produced in which the germinal material fills only a fraction of the cavity. Protoplasmic strands are present extending from the wall across the lumen and the germinal material is attached to these strands. Just how this relation develops is not clear, but the fact remains that in immature daughter sporocysts germ cells, cleavage stages, germ masses and even separate embryos are frequently found attached to the walls by such strands.

Germ masses are so numerous and consistently present in immature daughter sporocysts of *C. stagnicolae* that it seems probable that all the numerous germ cells present in the daughter at emergence go through this type of multiplication. Although a few germ masses and early embryos are still present in daughter sporocysts in which most of the cercarial embryos are approaching maturity, many of the germ masses develop synchronously and a large proportion of the cercarial embryos in an older daughter sporocyst are at about the same stage of development. Consequently, in infections from which large numbers of cercariae are escaping a large proportion of the sporocysts will contain only mature or almost mature cercariae. This tendency toward synchronicity in the polyembryony in the daughter sporocysts leads to the development of large numbers of cercariae over a rather limited period of time and a rather rapid exhaustion of the infection.

In the sporocyst stages of *C. stagnicolae*, therefore, an enormous multiplication of individuals is brought about both by a multiplication of germ cells in the embryonic development of the daughter sporocysts and by polyembryony by means of germ masses in both the mother and daughter sporocyst stages. A comparison of the germ cell cycle of *C. stagnicolae* with that of the strigeids (Cort and Olivier, 1941) will

make these relations clearer. In the strigeids small numbers of germ cells in the miracidium produce small numbers of germ masses in the mother sporocysts. These germ masses are discrete entities with their own membrane, which persist throughout the whole life of the mother sporocysts and produce daughter sporocyst embryos by the breaking off of their largest end components. Even in old mother sporocysts, which persist in infections in which most of the daughter sporocysts are mature, the germ masses show no signs of reproductive exhaustion and contain numerous unicellular components. Also, the daughter sporocysts of strigeids, when ready to emerge, contain only germ masses (about 12 to 24). In the migrating stage these germ masses grow larger and begin to give off cercarial embryos. No separate germ cells are present. These germ masses persist as discrete entities and produce cercarial embryos throughout the life of the daughter sporocysts. This means that in the strigeids a constant supply of cercarial embryos is produced and that new cercariae are developing and escaping at a relatively constant rate throughout the whole life of the infection. It can be seen, therefore, that the germ cell cycle of *C. stagnicola* differs chiefly in two particulars from that of the strigeids. In the first place its germ masses are not such discrete entities and the phases of multiplication by polyembryony, both in the mother and daughter sporocysts, seem to be more transient and irregular. In the second place, large numbers of separate germ cells are produced, especially in the development of the daughter sporocysts, before the germ masses appear.

In the few other descriptions of schistosome mother sporocysts we have found in the literature (Miyairi and Suzuki, 1914; Faust and Meleney, 1924; Price, 1931; and Wall, 1941) the contents of the body cavity are described as consisting of germ cells and early embryos in the germ ball stage. No structures that could be interpreted as germ masses were described or figured. In fact, in none of these accounts is the question of multiplication of the germinal elements even considered. Also, in the descriptions or figures of the early embryonic stages of the daughter sporocysts within the mother and of the immature stages in the snail's tissues accurate details are not provided. It seems clear, however, that the presence of spines on the anterior tip of young daughter sporocysts is probably a characteristic of the whole schistosome group since they have been described for *Schistosoma japonicum* by Miyairi and Suzuki (1914), for *S. mansoni* by Faust and Hoffman (1934), for *Schistosomatium douthitti* by Price (1931), and for *Spirorchis parvus* by Wall (1941). The birth pore of mother and daughter sporocysts has apparently not been noted for other schistosome species, although it seems probable that it is present. Certainly, on account of the lack of information on other schistosome species no generalizations on early sporocyst development and the course of the germ cell cycle can be made at the present time for the whole schistosome group.

SUMMARY

Studies were made from natural infections in *Stagnicola emarginata angulata* (Sowerby) of the mother sporocysts and the development of the daughter sporocysts of *Cercaria stagnicola* Talbot, 1936. The mother sporocysts of this schistosome are thin walled, sausage shaped sacs with an attenuated anterior end bearing a birth pore and canal. They are usually located in the tissues on the surface of the organs of the snail in front of the digestive gland, particularly on the kidney and stomach. In the

smallest mother sporocysts found, which were about 3 mm in length, the germinal material, consisting of single germ cells, groups of a few germ cells, irregular germ masses, and a few germ balls (immature daughter sporocyst embryos), is attached to protoplasmic processes from the body wall. In somewhat older sporocysts the contents, consisting of germ masses and daughter sporocyst embryos, float freely in the body cavity and few, if any, protoplasmic processes persist. The irregular germ masses, some with only multicellular components and others with both multicellular and unicellular components, frequently are still present in mature mother sporocysts. The developing daughter sporocyst embryos consist of masses of separate germ cells enclosed by thin walls of flattened cells. Even in the daughter sporocysts that are ready to escape from the mother germinal material consists only of numerous separate germ cells.

After the escape of the daughter sporocysts into the tissues of the snail there is a rapid increase in size and a stage when the germinal material fills only a fraction of the body cavity and is attached to protoplasmic processes from the wall. Numerous irregular germ masses are produced similar to those found in the mother sporocysts. In stages of daughter sporocysts approaching maturity the contents consist of large numbers of cercarial embryos which nearly fill the cavity and few, if any, germ masses persist.

In the sporocyst stages of *C. stagnicolae*, therefore, enormous cercarial production is made possible by the large numbers of germ cells that develop in the daughter sporocysts before they leave the mother and by phases of polyembryony by germ masses in both the mother and daughter sporocysts. The germ cell cycle of *C. stagnicolae* differs from that of the strigeids chiefly in two particulars, viz., (1) its germ masses are not such discrete entities and the phases of polyembryony, both in the mother and daughter sporocysts, appear to be more transient and irregular, and (2) large numbers of separate germ cells are produced, especially in the development of the daughter sporocysts, before the germ masses appear.

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THE MODIFICATION OF THE DIGESTIVE GLAND TUBULES IN THE SNAIL *STAGNICOLA* FOLLOWING PARASITIZATION

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As early as 1915 Cort noticed that the livers of the snail, *Lymnaea reflexa*, infected with *Cercaria douthitti*, were filled with a tangled mass of elongate, cylindrical sporocysts.

Faust (1917) suggested that a mechanical pressure of the larval stages of certain trematodes tended to affect the glandular organs by inhibiting or increasing their function and cramping the tissues within unusual confines.

Agersborg (1924) stated that the effect of such a trematode infection could be divided into four phases. There was a distorted and disintegrated condition of the tissues. The tissues reacted to the invasion by the production of black granules which were discharged into the intracellular spaces. A very distorted condition existed in the liver with the epithelium nearly destroyed, producing the effect of squamous rather than the normal columnar epithelium. If the snail survived there was a return to the normal condition.

Sometimes infected snails were abnormally large. Wesenberg-Lund (1934) first pointed out this fact. He advanced the idea that size might not be a criterion of the age of the snail, but that the excessive growth was due to the presence of parasites. This increase in size he attributed to the fact that the snails might have ingested abnormally large quantities of food in order to satisfy the demands of the parasites as well as themselves.

Rothschild has studied the phenomenon of gigantism in infected snails. In 1936 she recorded the relationship of large size and parasitism in the snail *Peringia ulvae*. In 1941 she determined by x-ray sterilization that the increase in size of the snails was not due to castration by the parasite. The abnormal size of the infected snails seemed to be a result of effects of the parasite upon the digestive gland of the snail.

Brackett (1940) found that snails brought into the laboratory often failed to emit cercariae until after they had been fed or recommenced after a starvation period.

Cort, Olivier, and McMullen (1941) stated that old infections of larval trematodes almost completely destroyed the digestive glands of the infected *Physa parkeri*. Their method of determining the destruction was not explained.

Pratt and Barton (1941) noted that the effect of a heavy infection was to reduce the apparent number of liver tubules and to limit them to the periphery of the liver area. The parasites were found in the central area of the liver or digestive gland and scattered between the liver tubules. No parasites were found in the centers of the liver tubules, the hermaphroditic duct, the ovotestis or the intestine.

The present work was a continuation of the work by Pratt and Barton. An effort was made to determine the effect of the infection upon the number and distribution of the liver tubules and upon their histology.

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MATERIALS AND METHODS

The snails studied were *Stagnicola emarginata angulata* (Sowerby). They were collected from Douglas Lake, Michigan. The cercariae from each snail were identified by Dr. Sterling Brackett while they were alive. The snail was then extracted from the shell and fixed in Bouin's or Gilson's fluid.

The fixed material was embedded in paraffin and serially sectioned 14 microns thick, stained in Semicon's carmine, dehydrated and mounted in damar.

The cercariae coming from the snails were identified as *Cercaria laruei* (Cort and Brooks, 1928), and *Diplostomum flexicaudum* (Cort and Brackett, 1937). All snails studied had been shedding only one kind of cercariae at the time that they were killed. A snail which was not shedding cercariae was studied for comparisons with the infected snails.

Each liver tubule that was large enough to have a lumen and each parasite that showed a distinct limiting membrane was counted in two snails, one infected with *Cercaria laruei* and the other that proved to be without parasites. A survey of these data indicated that counting the liver tubules and parasites in every tenth section gave a representative picture of the material. The remaining two snails studied were analyzed in this manner. The words digestive gland and liver are used synonymously in this paper.

DATA

The snail that was not parasitized (Snail A) was characterized by a digestive gland that tapered fairly smoothly at both ends. The twisted condition of the organ accounted for the fact that the posterior or spire end of the gland terminated abruptly as it did in two of the other snails. The maximum breadth of the gland occurred between the 100th and 200th sections which constituted the second fourth of the gland from the anterior end. The maximum number of tubules in any one cross section was 102. The total length of the digestive gland was about 5.0 mm, but this specimen was fixed in Gilson's fluid while the other ones were fixed in Bouin's. The Gilson's fluid caused the material to shrink more than did Bouin's.

Snail B was infected with the trematode *Diplostomum flexicaudum*. In the anterior end of the digestive gland there was sufficient room so that the parasites did not crowd the liver tubules. At about the hundredth section, which was approximately 1.4 mm back, the parasites became numerous. Fifty and more parasites were counted in each cross section. The liver tubules in this area differed from those in the corresponding area in the uninfected snail in that the number of them was reduced. Instead of this being the region where the tubules were the most numerous, as it was in the uninfected snail, the maximum number of tubules was found much farther posterior in the gland. They reached the greatest number at the beginning of the last fourth of the gland. As compared to the other snails studied this was the lightest infection. In no cross section were there as many as a hundred parasites. The number of parasites decreased toward the posterior or spire end of the digestive gland, but 3 were found in the last section.

Snail C was infected with *Cercaria laruei*. This was the heaviest infection of the three studied. The gland from the 50th to the 250th section contained large numbers of parasites, as many as 224 in one section. This part of the gland was the one in which the maximum number of tubules was found in the uninfected snail.

The effect of the parasites was apparently to spread out the liver tubules. At the upper end of the gland there were no parasites in Snail C. The gland was approximately 6.7 mm in length and constituted 480 cross sections.

TABLE 1.—Serial sections of the digestive glands of *Stagnicola emarginata angulata*. Comparison of number of digestive gland tubules and parasites present in every 10th section. Snail A not infected. Snail B infected with *Diplostomum flexicaudum*. Snails C and D infected with *Cercaria laruei*

Serial section	Snail A tubules	Snail B		Snail C		Snail D	
		Tubules	Parasites	Tubules	Parasites	Tubules	Parasites
1	10	2	37	17	19	3	14
10	26	4	28	14	40	8	12
20	25	14	24	21	58	14	19
30	27	24	16	23	74	26	19
40	29	31	17	28	78	35	27
50	29	32	22	26	96	31	39
60	41	33	17	26	111	40	41
70	64	38	24	38	64	44	48
80	68	49	33	29	95	52	56
90	50	52	41	32	79	49	57
100	77	38	50	23	118	24	56
110	70	30	52	29	90	34	79
120	102	33	60	49	224	39	107
130	84	19	65	62	127	24	88
140	82	28	84	74	176	24	91
150	79	29	91	60	100	25	85
160	84	37	77	50	44	37	116
170	77	60	92	96	114	37	109
180	87	51	83	83	167	51	112
190	77	70	86	95	166	68	86
200	65	47	70	58	164	65	92
210	48	57	65	60	99	69	86
220	65	98	64	68	92	66	102
230	62	82	75	72	87	62	110
240	45	74	85	63	80	35	139
250	52	87	83	60	59	36	143
260	50	63	87	64	61	52	156
270	31	63	88	55	69	25	154
280	21	62	78	60	54	39	120
290	19	77	84	59	65	30	132
300	24	81	98	66	65	42	123
310	30	90	81	70	48	49	110
320	14	87	90	69	31	45	85
330	13	104	91	80	28	52	78
340	17	77	89	78	17	43	73
350	24	78	77	78	16	43	44
360	12	70	74	65	19	42	45
370	17	58	64	66	13	43	33
380		42	57	63	17	34	50
390		45	33	63	8	31	59
400		36	16	57	10	38	63
410		36	3	75	11	39	61
420				52	11	32	35
430				59	6	30	35
440				46	7	28	31
450				43	1	29	22
460				13	0	34	18
470				17	0	38	23
480				16	0	19	22
490						16	18
500						21	30
510						27	28
520						18	39
530						18	37
540						16	26
550						5	32
560						11	9
570						5	3
578						0	2

Snail D, which was also infected with *Cercaria laruei*, showed the suppression of liver tubules in the part of the digestive gland where the parasites were most numerous in a distinct pattern. From section 100 to section 180 the parasites were numerous. In that part of the gland the liver tubules numbered in the twenties and thirties, although the number of tubules below the concentration of parasites was around 50 per microscopic section. The number of parasites fell off in the region of sections

190 to 210 and in the same region the number of tubules was in the sixties. Again at section 220 the parasites increased in number and were numerous back to section 310. Again the number of liver tubules dropped back into the twenties and thirties. The suppressing effect of the heavy parasitization was evident up to about 20 sections (0.28 mm) from the margin of the mass of parasites at both ends of the parasitic mass. The parasites and liver tubules gradually tapered off toward the spire end of the gland and ended almost together. The total length of this gland was about 8.1 mm.

The gland tubules of Snails A and D and the parasites in Snail D were counted for each section of the entire gland. A tubule appeared to extend for about ten sections. Therefore each tubule was counted a number of times. However the same procedure was followed in both snails. Snail A, which was not parasitized, contained 20,417 sections of liver tubules; Snail D, which was fairly heavily parasitized, contained 20,105 sections of liver tubules. The difference in number of tubules between the uninfected and the parasitized snail was insignificant.

The effect of the parasites upon the histology of the liver tubules was similar to that found by Agersborg (1924). In the immediate vicinity of the parasites the liver tubules were reduced from a columnar epithelium to a cuboidal or flattened type. In adjacent areas the unaffected columnar epithelium was characterized by a conspicuous layer at the lumen end of the cells. This layer was absent in the cells of tubules immediately adjacent to parasites. These latter cells did not stain as sharply as did the unaffected cells. Droplets within the columnar cells showed up very clearly in the preparations as empty spaces. These clear areas were also present in the flattened cells found adjacent to parasites. The delicate connective tissue network was much disrupted by the parasites as noted by Pratt and Barton (1941).

DISCUSSION

When the entire number of cross sections of Snail A were counted there was a fairly regular increase and decrease in number of liver tubules. Examination of the microscopic slides suggested that the gland branched something like a fir tree. The maximum number of tubules corresponded to the branches of the tree and the minimum between two maxima corresponded to the spaces between successive branches from the main tree stem. There was a main opening into the intestine just above the lower end of the gland and a main duct from which the secondary branches sprung.

Cort, Olivier and McMullen (1941) found that the livers of *Physa parkeri* were almost completely destroyed by the larval trematode infections. To the contrary, this present work did not disclose convincing evidence of destruction or decrease in amount of liver tissue.

The layer on the lumen end of the cells of unaffected columnar epithelium certainly resembled a secretory layer as seen in vertebrate glands. The tubules in the region of parasites showed an histological change toward a tissue that probably was not secreting, characterized by flattened cells making up the wall of the tubules. It is possible that the presence of the parasites caused the tubules to go through a secretory cycle and become exhausted. There might be a relationship between these histological data and the observations of Wesenberg-Lund (1934) and Rothschild

(1936, 1938, and 1941) that parasitized snails are larger* than are the snails of the same age and population that are free of larval trematodes.

Although the number of liver tubules was fewer in the parts of the gland where the parasites were most numerous, the evidence suggests that the tubules were merely displaced posteriorward. The data indicate that the infection had to be quite heavy in order to produce the regional reduction in number of liver tubules.

In preparing the digestive glands of snails for the study, several sets of slides were made of the remainder of the tissues of the snails. This technique was difficult because certain tissues became very hard and brittle and fell away from the paraffin ribbon. However, in those specimens where this fault did not occur, it was possible to get some idea of the relationship of the parasites to the other organs of the body of the snail. Although the parasites were not numerous, there were stages other than cercariae in the body cavity lying free among the organs.

SUMMARY

1. Four snails identified as *Stagnicola emarginata angulata* were fixed, serially sectioned, stained, mounted in damar and studied to ascertain the effect of larval trematodes upon the digestive gland.

2. Snail A was found to be free of trematode infection and was studied in detail to discover the normal distribution of liver tubules in the digestive gland of this species of snail, and the histological characteristics of the cells making up the tubules. The liver was formed something like a fir tree with the greatest bulk occurring in the second fourth from the anterior end of the gland. The cells making up the ultimate branchings of the gland constituted a columnar epithelium. A layer in these columnar cells adjacent to the lumen of the liver tubule appeared to be secretory in nature. Droplets, in addition to the layer, were found throughout the length of the cells.

3. Snail B was infected with *Diplostomum flexicaudum*. This infection was fairly light with never as many as a hundred parasites in a single cross section. The effect of the infection was to cause the maximum number of liver tubules to be found farther posteriorward than in the uninfected snail.

4. Snail C infected with *Cercaria laruei* harbored the heaviest infection of those studied. The greatest number of parasites was in the area from about one-tenth to one-half of the way back, and the liver had the most tubules about two-thirds of the way toward the spire end where the number of parasites was about one-sixth as great as farther forward.

5. In Snail D, which was infected with *Cercaria laruei*, the parasites were grouped into two main masses and the liver tubules were suppressed in both those regions, although the effect of the crowding did not show in the number of liver tubules for about 0.28 mm within the mass of parasites.

6. The total number of sections of liver tubules was counted in Snails A and D. The uninfected snail had 20,417 and the infected snail 20,105 sections of liver tubules. There had thus been no destruction of liver tubules in Snail D as judged by the tubule section counts.

* Rothschild (1938) was misquoted in the paper by Pratt and Barton (1941). In paragraph 4 of the latter the text should have read "... *Peringia ulvae* when parasitized by trematodes were larger than other individuals of the same age that were not parasitized."

7. The tubules in the vicinity of the parasites showed a modification of structure from cells of columnar type to cuboidal or squamous type. The unaffected cells not adjacent to parasites were like those in the non-parasitized snail.

8. Although there was no secretory layer on the affected cells, there were droplets within the flattened cells.

9. A possible relationship between the modifications of the cells adjacent to the parasites and the gigantism among parasitized snails was suggested.

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TWO NEW LARGE-TAILED CERCARIAE (PSILOSTOMIDAE) FROM NORTHERN MICHIGAN*

LESLIE R. HEDRICK

In the summer of 1941 the author studied some of the larval trematodes of the smaller snails of the Douglas Lake region in Michigan. The bottom vegetation and ooze where the snails abide was scooped up with a large galvanized pan provided with a brass screen bottom. This material containing the snails was placed in a wash basin and the snails were separated from the lighter debris by a series of decantations. This was usually done in the field. Rock pebbles were often with the snails after the other material had been removed. At the laboratory the snails and rocks were placed in a large, glass, flat-bottom container and swirled with water so that the snails and rocks were in the center of the dish. After a few hours, nearly all the snails had wandered toward the periphery of the dish free from the rocks. Then the snails were separated into genera and species. Ten to fifteen snails of a kind were placed in a Syracuse watch glass with enough water to cover the animals.

The next day these dishes were examined for the emergence of cercariae. Approximately 10,000 *Amnicola limosa* Say were collected, some of which gave emergence to three different large-tailed forms which probably belong to the psilostome group. The names *Cercaria ameeli* n. sp. and *Cercaria limosae* n. sp. are proposed for two of these. The other form was found only once and not enough data were recorded for an adequate description. Descriptions of the cercariae and rediae will follow. All the cercaria measurements were made from specimens killed in 4% formalin, heated to 50° C. The rediae were measured from living material.

Cercaria ameeli n. sp.

(Figs. 1-3)

Specific diagnosis: Psilostome cercaria (Fig. 1). Cuticular spines limited to anterior region of body, extending posteriorly only to level of middle of anterior sucker; remainder of body wall spineless but with thick granular cuticle. Oral sucker terminal and unspined, very muscular; approximately same size as acetabulum. Prepharynx rather long; pharynx pyriform and robust, more than one-half length of oral sucker. Esophagus clear, wider than pharynx. Intestinal ceca clear, branch just anterior to acetabulum and extend more than half way around acetabulum. Acetabulum near posterior end of body, large, concave, and protrudes extensively from ventral body surface. Excretory bladder two chambered; anterior, rectangular chamber in body empties into posterior, triangular chamber in tail by narrow tube. Major collecting ducts arise from antero-median margin of anterior chamber and quickly diverge posterior to acetabulum to extend in lateral field to near anterior end of body. Flame cell pattern, five pairs on either side; flame cells rather large. No penetration glands found but two "glandular" areas present in anterior end of body on either side of oral sucker and prepharynx. Posterior to these areas, body contains many polygonal cystogenous bodies with fine rod-like striations.

Tail is more than twice length of body and wider than body (Fig. 2); flattened and ribbon shaped with smooth margins when relaxed. Color of tail yellow-amber. Seven to nine pairs of clusters of large amber granules in two lateral areas of tail. These large granules not definitely attached to any structure but have limited range of flow in lymph of tail. Small yellow uniformly distributed granules diffusely scattered throughout tail. Triangular chamber of excretory bladder in anterior end of tail terminates in short blind tube.

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* Contribution from the University of Michigan Biological Station and the Biology Department, Illinois Institute of Technology.

Measurements of thirty specimens; mean in microns with its probable error; extremes in parenthesis. Body length 213 ± 2.5 (185–281); body width 99 ± 1 (77–122); tail length 447 ± 7.5 (339–585); tail width 121 ± 2.5 (90–145); oral sucker diameter 39 ± 2 (35–42); acetabulum diameter 40 ± 2 (35–48); distance of acetabulum to posterior end of body 22 ± 0.5 (14–28); prepharynx length 23 ± 1 (14–34); pharynx length 26 ± 0.5 (17–31); pharynx width 14.

Host: *Amnicola limosa* Say.

Locality: Douglas Lake, Cheboygan County, Michigan.

REDIAE OF *C. ameeli*

The redia of *C. ameeli* has a well-developed sucker and a very prominent pharynx. The intestine extends to the posterior end of the body and is a rich chestnut brown in color. The birth pore is just posterior to the pharynx (Fig. 4). The length of three rediae ranged from 0.9 to 1.2 mm. The width ranged from 0.22 to 0.26 mm. The length of the redial pharynx is 65μ ; the width of the pharynx is 52μ . The rediae were rather active. The tails of very young cercariae within the rediae are distinctly granular, but the granules are colorless and refractory. In more developed immature cercariae within the rediae, the granules are colored and much more numerous than in mature cercariae. Colorless granules surround the cercariae within the body of the redia.

Approximately fifteen *Amnicola limosa* of the 10,000 collected produced *Cercaria ameeli*. The daily output is thirty to fifty cercariae. The cercariae swim on their sides with the tail having the appearance of an undulating ribbon. The tail usually pushes but it may also pull the body. When the cercariae rest on the bottom of the dish, the tail is straight. Cercariae which are relaxed and suspended in the water tend to come to the surface. The tails of relaxed cercariae in a vial of water are straight and in a vertical position. These tails apparently support the smaller bent cercarial body which hangs below (Fig. 3).

Cercaria limosae n. sp.

(Figs. 5, 7)

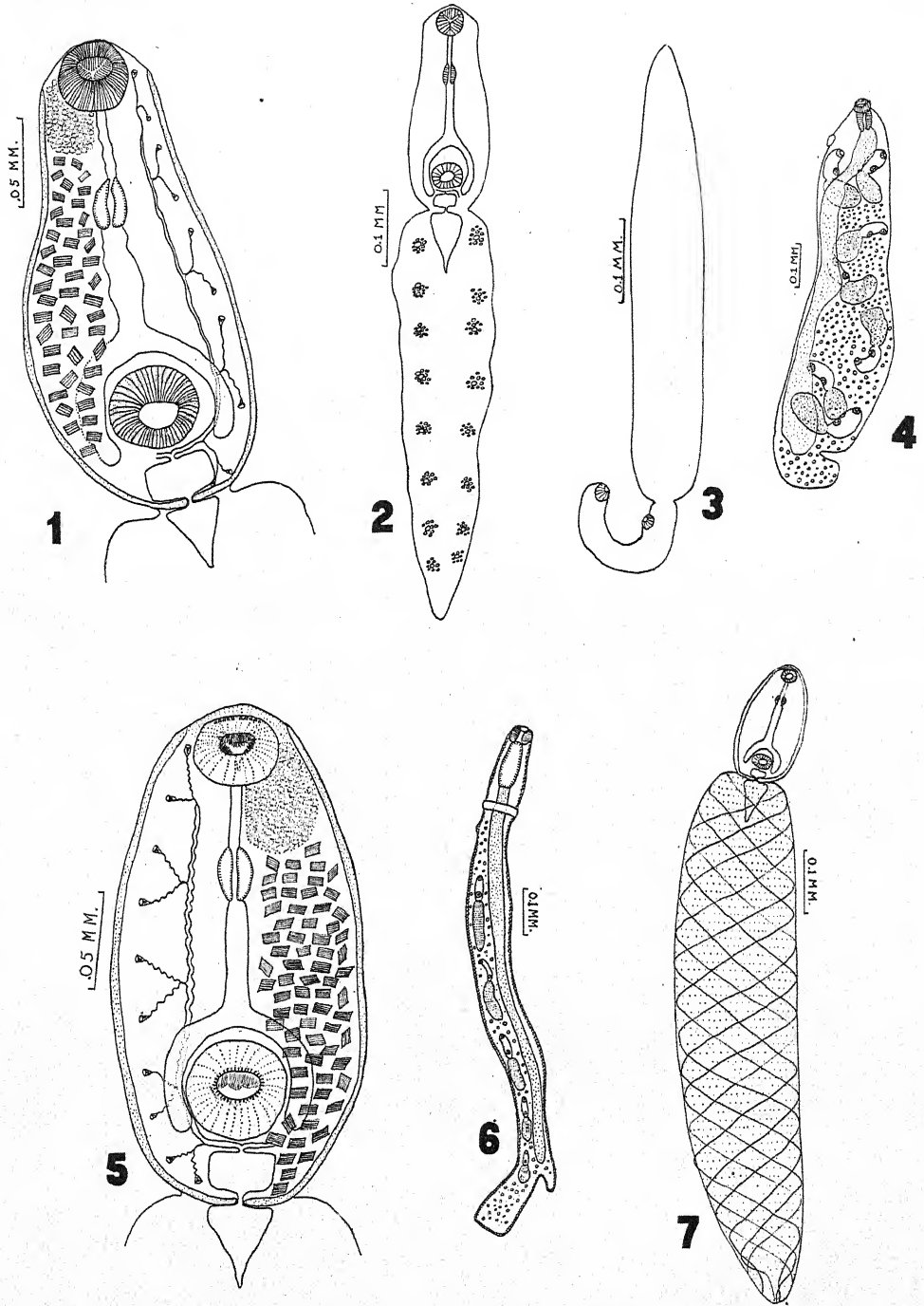
Specific diagnosis: Psilostome cercaria (Fig. 5). Cuticular spines limited to anterior region of body, extending posteriorly only to level of middle of anterior sucker; remainder of body wall spineless but with thick granular cuticle. Oral sucker terminal, very muscular with ten rather broad spines on anterior border; approximately same size as acetabulum. Prepharynx rather long; pharynx pyriform and robust, more than one-half length of oral sucker. Esophagus clear, wider than pharynx and extending posteriorly nearly to acetabulum, where it branches to form two clear ceca which extend more than half way around acetabulum. Acetabulum near posterior end of body; large, protrusible; distal margin with thirty-six rather broad spines. Excretory bladder two chambered; anterior, rectangular chamber in body empties into posterior, triangular chamber in tail by narrow tube. Major collecting ducts arise from antero-median margin of anterior chamber and quickly diverge posterior to acetabulum to extend in lateral field to near anterior end of body. Flame cell pattern five pairs on either side; flame cells rather large. No penetration glands found but two "glandular" areas present in anterior end of body on either side of oral sucker and prepharynx. Posterior to these areas, body has many polygonal cystogenous bodies with fine rod-like striations.

Tail rufous brown in color and appears reticulated with interlacing lymph canals (Fig. 7). Pigment in small granules uniformly distributed. Tail four to five times as long as body and nearly twice as wide. Triangular excretory chamber in anterior end of tail ends in a blind narrow sac. Cercaria swims on its side and tail in swimming motion appears to be undulating ribbon.

Measurements of fifteen specimens; mean in microns with its probable error; extremes in parenthesis: body length 228 ± 1.5 (195–270); body width 107 ± 1.5 (93–124); tail length 833 ± 34.5 (570–1050); tail width 202 ± 6.5 (155–248); oral sucker diameter 42 ± 1 (37–56); acetabulum diameter 44 ± 0.6 (40–55); distance of acetabulum to posterior end of body 21 ± 0.7 (16–28); prepharynx length 23 ± 1 (18.5–26); pharynx length 23 ± 0.6 (19–31).

Host: *Amnicola limosa* Say.

Locality: Douglas Lake, Cheboygan County, Michigan.



During the course of the summer, five *Amnicola limosa* were found to be infected with *Cercaria limosae*. Each snail releases approximately ten cercariae per day. However, during the warm weather the snails only lived in the laboratory two or three days and seldom did I have over one infected snail at any one time, so I was unable to carry out any extensive feeding experiments. When the cercariae relax, the tail is straight and flat. This species was not observed in a vertical container, so I am unable to report whether these forms hang in the water with the tail upward and the body downward.

REDIAE OF *C. limosae*

The redia has a collar, a well-developed sucker and a large pharynx (Fig. 6). Birth pore posterior to collar. Intestine, which extends to near the posterior end, filled with chestnut brown granules. Epithelioid layer just under the body covering, amber in color. Caudal appendages near posterior end. Length of mature rediae 1.5 to 2.7 mm. In the developing cercariae, the posterior apex of the tail portion of the bladder extends in a median line two-thirds the length of the tail. Here it divides into two branches, each of which opens to the exterior on the sides of the tail. In the fully-developed cercariae, both before and after emergence, this posterior extension from the apex of the tail portion of the bladder is absent.

INFECTION EXPERIMENTS

Several series of experiments to determine the second intermediate host for *C. ameeli* were negative. Small fish or tadpoles were placed in a finger bowl containing a known number of cercariae (usually 40 to 100). In several instances of direct observation with a binocular dissecting microscope, the cercarial body and tail were drawn into the mouth or external nares with the respiratory current. The suspected host was left with the cercariae for several hours or overnight. Then the number of remaining cercarial bodies and cercarial tails was determined in order to ascertain the number of cercarial bodies in the experimental host.

In ten such experiments with young *Perca flavescens*, no metacercariae were found either after an interval of one or two days, or after a three-week interval. In all of these perch, 25 to 50 cercarial bodies were determined to have entered. Likewise, results were negative when young bullheads, guppies and tadpoles were used. In experiments with *C. limosae*, the methods used were identical with those described for *C. ameeli* except that I worked with smaller numbers of cercariae, namely ten to twenty. The results were also negative.

DISCUSSION

A comparison of the two forms described above with the cercariae of the four life histories described for the psilostome group reveals general relationships, but

FIG. 1. Body of *Cercaria ameeli*, anterior glandular area and cystogenous glands show on one side; flame cell arrangement on the other side.

FIG. 2. Outline of entire *Cercaria ameeli*, showing location of large granules in tail.

FIG. 3. *C. ameeli* in natural resting position in water.

FIG. 4. Redia containing young *C. ameeli*.

FIG. 5. Body of *C. limosae* with flame cells shown on one side and glandular area and cystogenous glands on the other side.

FIG. 6. Redia containing young *C. limosae*.

FIG. 7. Outline of entire *Cercaria limosae* showing reticular character of tail due to inter-lacing lymph canals.

also some differences. Life histories for four Psilostomes have been described—*Psilotrema spiculigerum* (Mühling) by Mathias (1925), *Sphaeridiotrema globulus* (Rud.) by Szidat (1937), *Psilostomum ondatrae* Price by Beaver (1939) and *Psilostomum reflexae* (Cort) by Feldman (1941). In the cercariae of all of these forms, there is a definite excretory siphon which recurves backward near the anterior sucker. In the forms just described, no siphon was found and the collecting ducts did not recurve backward and neither did they contain granules.

The flame cell patterns for the cercariae of *Psilotrema spiculigerum* and *Sphaeridiotrema globulus* were not described. In the cercariae of *Psilostomum ondatrae*, as reported by Beaver, the flame cells were in eight groups of three on each side of the body. Feldman described the flame cell arrangement for *Psilostomum reflexae* as forty-two pairs. The flame cell pattern for both *C. ameeli* and *C. limosae* is five groups of two on either side. For general appearance and swimming action, the cercariae I have described compare very well with the cercariae of *S. globulus* as described by Du Bois for *C. helvetica* XVII, 1928 and Szidat, 1937.

The arrangement of the excretory bladder and the shape and general structure of the tail of my forms are somewhat similar to those described for the "amphistome cercaria" of Peterson, 1931, and *Cercaria obscura* of Wesenberg-Lund, 1934.

SUMMARY

Two new large-tailed psilostome cercariae, *C. ameeli* n. sp. and *C. limosae* n. sp., and their respective rediae are described from *Annicola limosa* Say of the Douglas Lake Region.

Infection experiments upon young perch, young bullheads, guppies and tadpoles to determine the second intermediate hosts were negative.

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THE OCCURRENCE OF BLOOD PARASITES IN BIRDS FROM SOUTHWESTERN UNITED STATES¹

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A preliminary report on blood parasites of birds in the Southwest was published in 1937 by Wood and Wood. The present contribution includes the bird material of the earlier paper and therefore supplements that report. The slides that constitute the basis of this study were prepared at various times from November, 1935 through August, 1939. Most of the blood smears were made in 1937 and 1938. The number of slides prepared by each cooperator is as follows: William G. Webb (1), Walter Nichols (22), Professor Loye H. Miller (45), Carlos Stannard (58), P. J. Van Huizen (70), Josephine Michener (588), and F. D. and S. F. Wood (741).

MATERIALS AND METHODS

Most of the bird blood smears used in this study were made from banded birds by piercing the brachial vein on the under side of the left wing with a surgical needle. Others were made from gunshot wounds or internal organs. Therefore, barring natural destruction, most of these birds are still in the field. Band numbers of infected birds will be furnished other investigators on request to the senior author. The smears were air dried and stained in the laboratory with Jenner-Giemsa stain from one to many days after preparation.

All stained blood smears were examined for at least 20 minutes: 1 to 5 minutes with low power (6× oculars and 16 mm stirrup objective) and 15 minutes with the oil immersion lens (6× oculars). A count of the number of blood cells on a smear 1 cell thick during a 15-minute search with oil reveals at least 220,000 blood cells inspected. The actual sample count made on a negative slide involved the survey of 996 oil immersion fields in the 15 minute period with 225 cells per field (average of 10 fields) for a total of 224,100 cells. Therefore, for every bird reported here, approximately this number of cells was examined on at least one slide.

OBSERVATIONS

Examination of blood smears from 1,525 birds revealed 357 or 23.4 per cent infected with *Trypanosoma*, *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, *Hepato-*

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Success in finding parasites readily in smears is in some measure dependent upon the quality of the blood smear received. Special commendation in this regard is hereby expressed to Josephine Michener, P. J. Van Huizen and Loye H. Miller. The writers are indebted to Mr. George Willett of the Los Angeles Museum and Dr. Alden H. Miller of the University of California for advice on ornithological problems and to Dr. Fae D. Wood for her skillful execution of the drawings.

TABLE 1.—The relative incidence of blood parasites in southwestern birds

Common name	Scientific name	Number examined	Number infected	T.	HAEM.	P.	L.	HEP.	ILP.	M.	Infected localities
Anthony green heron	<i>Eurides virescens anthonyi</i>	1	1	1	...	1	1	Pasadena, Calif.
Crackling goose	<i>Branta canadensis minima</i>	5	1	1	Willows, Calif.
White-fronted goose	<i>Anser a. albifrons</i>	15	1	1	Willows, Calif.
Common mallard	<i>Anas p. platyrhynchos</i>	24	9	2	1	...	2	1	Willows, Calif.
American pintail	<i>Dafila acuta texithoa</i>	17	2	2	Willows, Calif.
Redhead	<i>Nyroca americana</i>	1	1	1	1	Pt. Mugu, Calif.
Red-breasted merganser	<i>Mergus serrator</i>	6	4	4	Pasadena, Calif.
Sharp-shinned hawk	<i>Accipiter velox</i>	12	6	...	6	Eagle Rock (1), and Pasadena (5), Calif.
Valley quail	<i>Lophortyx californica vallicola</i>	33	14	...	14	Los Serranos Game Farm, Chino, Calif.
San Quintin quail	<i>Lophortyx californica plumbea</i>	24	13	...	13	Phoenix, Ariz.
Gambel quail	<i>Lophortyx gambelii</i>	1	1	...	1	Mt. Diablo, Calif.
Mountain quail	<i>Oreortyx picta</i>	1	2	...	1	...	1	Piedra Blanca Spring, Ariz., and Pasadena, Calif.
Band-tailed pigeon	<i>Columba fasciata</i>	27	26	...	26	...	4	Pasadena (4), Calif., and Phoenix (22), Ariz.
Western mourning dove	<i>Zenaidura macroura marginella</i>	25	1	1	Pasadena, Calif.
Chinese spotted dove	<i>Spilopelia chinensis</i>	12	4	1	4	1	Phoenix, Ariz.
Western white-winged dove	<i>Melospiza asiatica meunsi</i>	1	1	1	1	1	Piedra Blanca Spring, Ariz.
Spotted screech owl	<i>Otus trichopsis</i>	1	1	1	1	Liebre Mts., Calif.
California spotted owl	<i>Strix o. occidentalis</i>	1	1	1	Pasadena, Calif.
Eastern flicker	<i>Colaptes auratus</i>	3	1	1	Pasadena, Calif.
Western flycatcher	<i>Empidonax d. difficilis</i>	20	10	3	9	Pasadena (9), and Los Angeles (1), Calif.
California jay	<i>Apelocoma o. californica</i>	3	1	Pasadena, Calif.
San Diego titmouse	<i>Baselophus inornatus transpositus</i>	1	1	Pasadena, Calif.
Pallid wren-tit	<i>Chamaea fasciata leishanet</i>	2	1	Pasadena, Calif.
Rock wren	<i>Salpinctes obsoletus</i>	61	10	4	4	San Nicolas Island, Calif.
Western mockingbird	<i>Mimus polyglottus leucopertus</i>	14	2	Pasadena (9), and San Fernando (1), Calif.
California thrasher	<i>Turdus migratorius propinquus</i>	1	1	Pasadena, Calif.
Western robin	<i>Hylocichla ustulata</i>	3	1	1	1	...	1	1	Shaver's Well, Calif.
Hermist thrush	<i>Hylocichla u. ustulata</i>	3	1	1	1	...	1	1	Berkeley, Calif.
Russet-backed thrush	<i>Vermivora celata</i>	5	1	1	Pasadena, Calif.
Orange-crowned warbler	<i>Vermivora celata</i>	2	1	1	Pasadena, Calif.
Calaveras warbler	<i>Dendroica aestiva</i>	19	4	3	Chiricahua Mts., Ariz.
Olive warbler	<i>Dendroica aestiva</i>	46	14	3	2	...	8	1	Pasadena, Calif.
Yellow warbler	<i>Dendroica auduboni</i>	1	1	1	1	Pasadena, Calif.
Audubon warbler	<i>Dendroica auduboni</i>	1	1	1	Borrego, Calif.
Black-throated gray warbler	<i>Oporornis tolmiei</i>	1	1	1	Pasadena, Calif.
Macgillivray warbler	<i>Icteria virens</i>	1	1	1	Pasadena, Calif.
Chat	<i>Wilsonia pusilla</i>	9	2	1	1	Pasadena, Calif.
Pileolated warbler	<i>Passer domesticus</i>	41	3	2	1	...	Pasadena, Calif.
English sparrow	<i>Icterus cucullatus</i>	18	4	4	11	2	2	Pasadena, Calif.
Hooded oriole	<i>Icterus bullockii</i>	76	6	3	4	...	1	Pasadena, Calif.
Bullock oriole	<i>Euphagus cyanocephalus</i>	55	7	2	...	3	3	Pasadena, Calif.
Brewer blackbird	<i>Molothrus ater obscurus</i>	16	12	4	9	2	1	...	Pasadena, Calif.
Western tanager	<i>Piranga ludoviciana</i>	46	34	5	24	...	18	1	...	1	Pasadena, Calif.
Dwarf cowbird	<i>Hedymeles melanocephalus</i>	2	2	1	Pasadena, Calif.
Black-headed grosbeak	<i>Passerina amoena</i>	2	2	2	Chiricahua Mts., Ariz.
Lazuli bunting	<i>Esperiphona vespertina</i>	2	2	Pasadena, Calif.
Evening grosbeak	<i>Carpodacus purpureus frontalis</i>	530	63	1	13	51	Pasadena (46), and Los Angeles (23), Calif.
California purple finch	<i>Carpodacus mexicanus frontalis</i>	2	2	...	2	San Nicolas Island, Calif.
Common house finch	<i>Carpodacus mexicanus clematis</i>	23	5	4	Pasadena, Calif.
San Clemente house finch	<i>Spinus tristis salicamans</i>	111	47	1	...	1	3	Pasadena (46), and Los Angeles (1), Calif.
Willow goldfinch	<i>Pipilo fuscus tristis</i>	41	1	...	23	...	30	Pasadena, Calif.
Gambel sparrow	<i>Zonotrichia leucophrys gambelii</i>	1	1	1	...	1	Pasadena, Calif.
San Diego song sparrow	<i>Melospiza melodia cooperi</i>	1427	357	42	159	71	133	8	7	13	Pasadena, Calif.
Totals											

Legend: T = *Trypanosoma*, Haem. = *Haemoproteus*, P. = *Plasmodium*, L. = *Leucocytozoon*, Hep. = *Hepatozoon*, ILP. = *Intra-leucocytic Parasite*, M. = *Microfilaria*.
 * Infected with *Spirogyra fusiformis*.

zoön, an intra-leucocytic organism, microfilarial worms, and a new parasite, *Spirogregarina fusiformis*, described below.

This survey included 112 species and subspecies of birds of which 54 species and subspecies or 48.2 per cent were infected. Credit for the extensive list of species is due entirely to the efforts of Josephine Michener and Loye H. Miller. Because of a shift of microscope equipment during this study, several light infections previously detected could not be verified even after extensive search of the slides. Therefore, the 23.4 per cent infection represents a minimum for the birds studied here. The data in Table 1 summarizes our findings. The birds are listed in the order in which they appear in the 1931 edition of the American Ornithologists' Union check-list.

Trypanosoma

Trypanosomes were found in 42 birds of 22 species or 11.8 per cent of the parasitized birds as shown in Table 1. These can be grouped somewhat as to type on the basis of morphology but no satisfactory species diagnosis can be made other than *Trypanosoma avium* Danilewsky until information is recorded on their life histories.

Haemoproteus

The genus *Haemoproteus* was observed in 159 birds of 21 species and subspecies or 44.5 per cent of the infected birds. *Haemoproteus lophortyx* O'Roke was found in the valley quail, San Quintín quail, Gambel quail and plumed quail. The great diversity of form characterizing this species creates the question of whether or not we are dealing with only one species in these birds. *Haemoproteus columbae* Kruse (= *maccallumi*) was found in the western mourning dove, western white-winged dove and the band-tailed pigeon. *Haemoproteus sacharovi* Novy and MacNeal was found in the western mourning dove and western white-winged dove.

The *Haemoproteus columbae* of the mourning dove showed gametocytes with irregular ends or regular, rounded ends. The latter structure was very similar to the gametocytes of *Haemoproteus* found in Audubon warbler, black-throated gray warbler, hooded oriole, Bullock oriole, western tanager, black-headed grosbeak, common house finch, San Clemente house finch, and Gambel sparrow.

A more robust type of *Haemoproteus* was seen in the spotted screech owl, the common mallard, and the American pintail.

Plasmodium

Parasites of the genus *Plasmodium* were found in 71 birds of 11 species or 19.9 per cent of the infected birds. The species encountered and their relative abundance in different kinds of birds is indicated in Table 2.

Leucocytozoon

Leucocytozoon were found in 123 birds of 33 species or 34.4 per cent of the infected birds.

Leucocytozoon simondi Mathis and Leger was found in 1 cackling goose, 1 white-fronted goose, 2 common mallards, 7 American pintails, 2 redheads, and 1 red-breasted merganser.

Leucocytozoon ziemanni (Laveran) was found in 1 California spotted owl. In 4 sharp-shinned hawks a parasite was found in which the cytoplasm of the host cell

was conspicuously short-tailed and whose host cell nucleus was less elongate than those parasitized by gametocytes of *Leucocytozoon simondi*.

Leucocytozoon in various stages of development characterized by the presence of a rounded form were seen in 1 Anthony green heron, 1 band-tailed pigeon, 4 western mourning doves, 1 Chinese spotted dove, 9 California jays, 4 western mockingbirds, 1 California thrasher, 1 western robin, 1 hermit thrush, 1 russet-backed thrush, 1 orange-crowned warbler, 1 Calaveras warbler, 1 olive warbler, 3 yellow warblers, 8 Audubon warblers, 1 Bullock oriole, 3 Brewer blackbirds, 3 dwarf cowbirds, 2 western tanagers, 18 black-headed grosbeaks, 1 lazuli bunting, 2 evening grosbeaks, 5 common house finches, 1 willow goldfinch, and 30 Gambel sparrows.

Hepatozoon

Hepatozoon was found in 8 birds of 6 species or 2.2 per cent of the infected birds. The organism occurred most frequently in lymphocytes and monocytes, tending to occupy most of the space available in the cytoplasm. Occasionally the position of the parasite resulted in a deep indentation on one side of the nucleus. The parasite

TABLE 2.—*Species of Plasmodium from southwestern birds*

Common Name	E	C	R	U	C & R
Anthony green heron	1
Western white-winged dove	1	..
California Jay	1	2	..
Western mockingbird	1	2	1	..
English sparrow	1	1	..
Hooded oriole	2
Brewer blackbird	1	2	..
Dwarf cowbird	1	1	..
Common house finch	14	19	16	2
California towhee	1
San Diego song sparrow	1
Total	1	15	29	24	2

Legend: E=*elongatum*, C=*cathemerium*, R=*relictum* (*praecox*), U=Unidentified, and C & R = *cathemerium* and *relictum*.

had a characteristic elongate, spindle-shaped form as seen here in organisms from the San Diego titmouse, common house finch, and pallid wren-tit.

The nuclear chromatin was in strings, masses or granules dispersed band-like across the middle of the parasite's body, thus separating the two ends of the cell. In the San Diego titmouse the nuclear chromatin was definitely granular (Fig. 14), the granules merging into loose strands but sometimes forming irregular moniliform bands or in some specimens appearing as a band-like mass across the parasite (Fig. 13). In the pallid wren-tit most parasites showed a definitely banded nucleus (Fig. 18) although in some the chromatin appeared in coarse clumps.

The cytoplasm appeared finely granular or, as in the San Diego titmouse, definitely vacuolated giving the protoplasm an alveolar appearance. A few small pink-staining granules were seen in the cytoplasm having the same color as the nuclear material of the parasite.

In one end of the cell there was a circular vacuole (or less often two vacuoles = excretory ?) which had a very uniform, finely granular appearance (Figs. 13, 17). The substance of the vacuole seemed to shrink away from the vacuolar membrane creating a narrow lighter zone between the vacuolar substance and its membrane. There was usually only one vacuole per parasite (Fig. 11) and it was closely pressed to the side of the nucleus and separated from the end and sides of the cell membrane by alveolar protoplasm. In the San Diego titmouse (Figs. 13, 14), the vacuole was set off sharply from the surrounding cell cytoplasm.

The host cell nucleus was somewhat flattened against the parasite but usually not indented.

There were no purple-stained granules of variable size in the cytoplasm of *Hepatozoon* such as were seen in the intra-leucocytic parasites discussed below.

The single parasite from the California thrasher (Fig. 16) resembled *Hepatozoon* in internal structure but its shape was more like that of the intra-leucocytic parasites.

Two parasites found in an Audubon warbler (Figs. 11, 12) were in thrombocytes and the granular nucleus of the parasite tended to occupy a transverse, band-like zone near the middle of the cell.

Intra-Leucocytic Parasites

Similar organisms were figured by Hewitt (1940) from the common house finch. Parasites of this type were found in 7 birds of 4 species or 2.0 per cent of the parasitized birds studied here. Hewitt erroneously ascribed to this category a *Toxoplasma* parasite described by Wood and Wood (1937) from this species of bird. However, additional comparative material has revealed Fig. 12 of Wood and Wood (1937) to be a developmental stage of *Leucocytozoon* in the common house finch.

The intra-leucocytic parasite from the western mockingbird (Figs. 9, 10), English sparrow (Fig. 4), dwarf cowbird (Fig. 5), and common house finch (Figs. 6, 7, 8) was a small round or oval, intra-lymphocytic organism. All parasites appeared to be at about the same stage in development. There was a definitely pink-stained nuclear mass which occupies a central position. The pale-staining nucleus may be round, elongate or dispersed across the cell in a band-like form. It appeared to be very variable in shape but consisted of coarse chromatin blocks with intertwining narrow chromatin strands and narrow parachromatin spaces. In some cases, as when the intra-leucocytic parasite was turned on edge from being pushed to one side of the host cell (Figs. 5, 7), the nuclear structure appeared more compact and homogeneous resembling Fig. 456 (6-12) of *Hepatozoon* as seen in Wenyon (1926).

The cytoplasm of the parasite appeared uniformly vacuolated suggesting an alveolar pattern. In some organisms practically no structure was visible except a pale, fine granulation of variable density giving the cytoplasm a mottled appearance. In free parasites, frequently small clear vacuoles were seen. These were occasionally found in the intra-lymphocytic forms.

In the cytoplasm, there were few to many, fine or coarse, dark purple-staining granules which tended to clump about the nucleus and were sometimes superimposed upon it. Hewitt (1940) seemed to have confused these cytoplasmic granules with chromatin which always has a light pink tint in our preparations. However, some of the smaller granules do resemble the nuclear chromatin in color and their tendency to clump about the nucleus aids in their being confused with it. These cytoplasmic granules were large and distinctly purple in our preparations from the western mockingbird and common house finch in contrast with the pale pink of their nuclei.

The host cell nucleus showed slight to pronounced indentation where in contact with the parasite.

In this study, the chief differences noted between *Hepatozoon* and the intra-leucocytic parasites were: elongate spindle-shape vs. a round or oval form, presence or absence of purple-stained cytoplasmic granules, band-like nucleus vs. round or elongate nucleus, and presence or absence of a distinct large cytoplasmic vacuole.

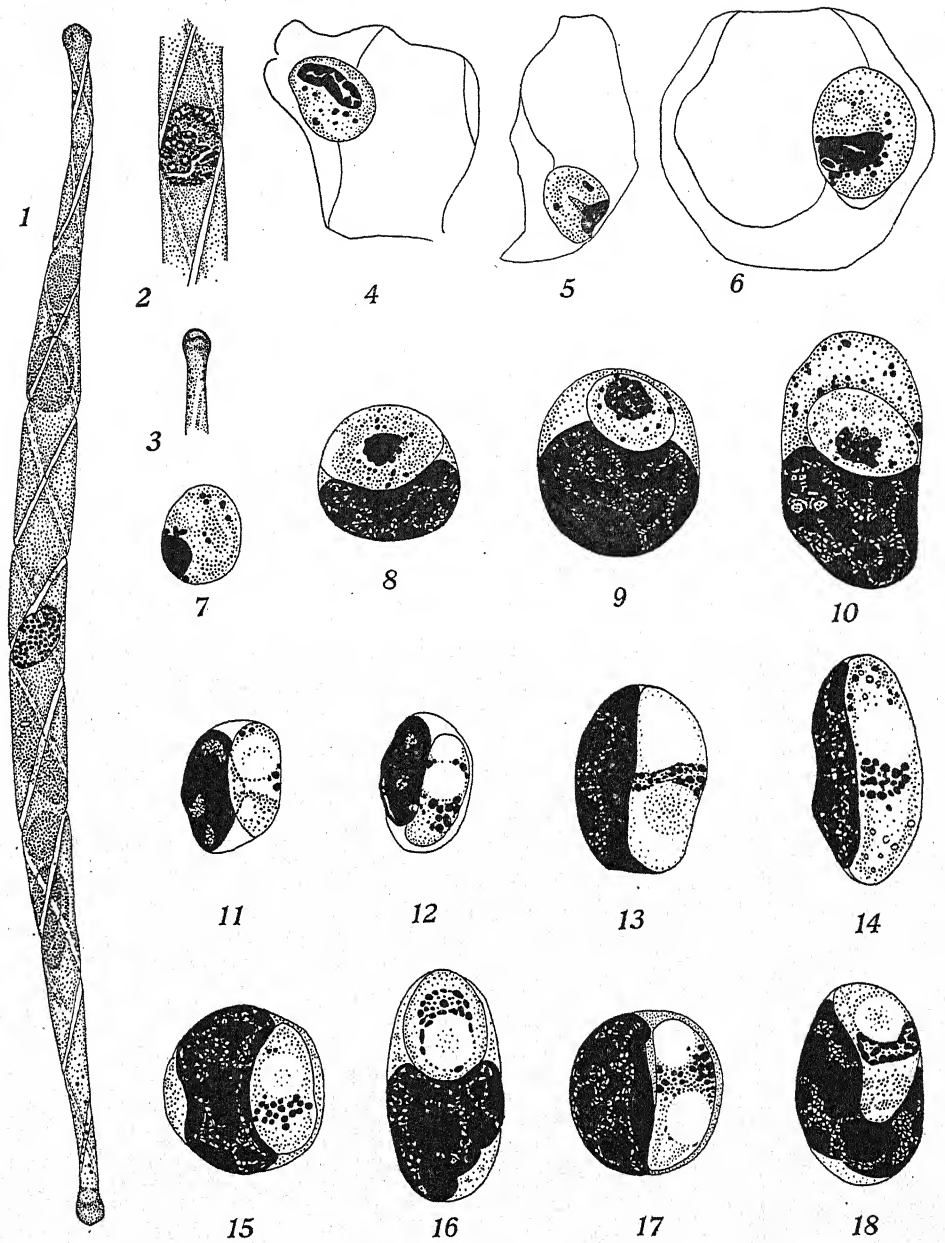
Figs. 1-3, $\times 1500$; Figs. 4-18, $\times 2588$

FIG. 1. *Spiroregarina fusiformis* n. g., n. sp., from the San Nicolas rock wren showing typical structure.

FIG. 2. Nuclear area of another *Spiroregarina fusiformis* showing irregularly-shaped nucleus surrounded by coarse granular mass.

FIG. 3. One extremity of another *Spiroregarina fusiformis* showing bulbous end suggesting a flattened or indented surface.

FIG. 4. Intra-leucocytic parasite in a monocyte from an English sparrow. Crushed host cell nucleus (to the right) and cytoplasm outlined.

FIG. 5. Intra-leucocytic parasite in a medium-sized lymphocyte from a dwarf cowbird. Host cell nuclear outline is to the right.

Microfilaria

Microfilarial worms were found in 13 birds of 10 species or 3.6 per cent of the infected birds. The total number found on one blood smear and the approximate size of single specimens is as follows: common mallard, 9 unsheathed forms ($104\ \mu \times 4\ \mu$); red-breasted merganser, 5 unsheathed forms ($296\ \mu \times 6\ \mu$); California spotted owl, 247 sheathed forms ($242\ \mu \times 8\ \mu$) and 175 unsheathed forms ($92\ \mu \times 5\ \mu$); western robin, 1 sheathed ($140\ \mu \times 4\ \mu$); russet-backed thrush, 336 unsheathed ($153\ \mu \times 4\ \mu$); pileolated warbler, 8 sheathed ($94\ \mu \times 4\ \mu$); hooded oriole, 4 unsheathed forms ($39\ \mu \times 3\ \mu$) in one bird and 135 unsheathed forms ($56\ \mu \times 4\ \mu$) in another bird; black-headed grosbeak, 2 sheathed forms ($118\ \mu \times 3\ \mu$); evening grosbeak, 8 unsheathed forms ($69\ \mu \times 4\ \mu$); and Gambel sparrow, 2 sheathed forms ($117\ \mu \times 4\ \mu$) in one bird, 22 unsheathed ($88\ \mu \times 4\ \mu$) in a second bird, and 27 unsheathed forms ($87\ \mu \times 3\ \mu$) in a third bird.

Spiroregarina fusiformis n. g., n. sp.

(Figs. 1-3)

Morphology: Elongate, spindle-shaped organism (Fig. 1), greatest width in center of cell where nucleus located. Body form in most specimens cylindrical; some individuals show flattening of extremities manifested by a twisting across itself of that portion of body. Most parasites straight in thick or thin parts of smear. On one slide an organism was found bent into half-moon shape. Ends of parasite flare to produce bulbous, knob-like terminus at each end (Figs. 1, 3). Outer surface of these expanded ends sometimes seems indented forming half-moon shaped flattened area (Fig. 3). Thus, parasite tapers gradually from middle nuclear zone of uniform width to neck-like constriction short distance from bulbous end, then flares out slightly towards rounded ends of cell.

Outer surface of parasite appears stiff but elastic suggesting presence of pellicle. Surface lined (grooved?) with light appearing, criss-cross bands of varying width running length of parasite in spiral arrangement. At least one spirally arranged band in clockwise direction and one in counter-clockwise direction.

Nucleus rounded and very compact in some individuals (Fig. 1); in others appears flattened. Frequently irregular in shape. Chromatin in dense globular masses which merge into each

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- FIG. 6. Intra-leucocytic parasite in a monocyte from a common house finch.
 FIG. 7. Free intra-leucocytic parasite from a common house finch.
 FIG. 8. Intra-leucocytic parasite in a small lymphocyte from a common house finch.
 FIG. 9. Intra-leucocytic parasite in a medium-sized lymphocyte from a western mockingbird.
 FIG. 10. Intra-leucocytic parasite in a large lymphocyte from a western mockingbird.
 FIGS. 11 and 12. *Hepatozoon* in thrombocytes from an Audubon warbler.
 FIG. 13. *Hepatozoon* in a small lymphocyte from a San Diego titmouse.
 FIG. 14. *Hepatozoon* in a small lymphocyte from another San Diego titmouse.
 FIG. 15. *Hepatozoon* in a small lymphocyte from a black-headed grosbeak.
 FIG. 16. *Hepatozoon* in a medium-sized lymphocyte from a California thrasher.
 FIG. 17. *Hepatozoon* in a small lymphocyte from a common house finch.
 FIG. 18. *Hepatozoon* in a small lymphocyte from a pallid wren-tit.

As seen on blood smears stained with Jenner-Giemsa, the nuclei of Figs. 1 and 2 appear purplish-red like the finer cytoplasmic granules while the coarser cytoplasmic granules around the nucleus (Fig. 2) and near the cell extremities are dark purplish-red. The striations vary from colorless to pink while the inclusion bodies have a reddish purple or reddish blue cast. The finely granular cytoplasm is reddish pink.

The black nuclei of the parasites in Figs. 4 through 10 are pale pink. The cytoplasmic granules vary from pink to purplish red and in some cells are almost black. The cytoplasm varies from pale blue to dark blue. The mottled appearance of the cytoplasm of the parasites, Figs. 4 through 18, is illustrated here with a fine stippling which does not represent the very fine cytoplasmic granulation.

The middle, band-like nuclear areas of the parasites in Figs. 11 through 18 vary from pale pink to purplish-red. The cytoplasm appears pale blue or gray-blue. The granules outside the nucleus are pale pink in some parasites or reddish purple to black in others. The vacuoles are usually colorless but appear pale blue in dark preparations.

other giving nucleus closely beaded appearance. In at least two specimens no distinct purplish-red staining nucleus found. In a few parasites, nucleus showed distinct bluish-staining nucleolus. Nucleus of number of specimens embedded in granular mass of material of similar structure to that of nucleus (Fig. 2). This granular mass tended to surround nucleus. Frequently, nucleus partly hidden by large, deep bluish-staining, oval bodies in cytoplasm.

Cytoplasm of parasite finely granular giving distinctly homogeneous appearance to lightly-stained individuals. Sometimes small unequal-sized, clear vacuoles in cytoplasm with especially thick membranes. In some cells, a number of coarse, deep purple-red staining granules distributed toward either extremity of cell, around nucleus, or along lighter staining spiral bands. In a few parasites these larger granules found throughout cytoplasm. In most parasites cytoplasm contained deep bluish-staining, oval masses (Fig. 1), so numerous as to completely fill central cytoplasm of cell. Oval masses sometimes showed two distinct parts, outer lighter homogeneous zone and central darker, less homogeneous area. Such structures similar in size and shape to red blood cells of host. Suggests source of food of parasite although structure of all parasites does not clearly indicate nature, or possibility, of ingestion apparatus.

Size: Average length of ten parasites chosen at random: $106.6\ \mu$ ($96.3\ \mu$ – $113.7\ \mu$). Diameter at widest central point averaged $4.7\ \mu$. Narrowest point of constriction before the end averaged $1.2\ \mu$ and diameter of the expanded end averaged $1.8\ \mu$.

Host: The parasite was found in one adult rock wren, *Salpinctes obsoletus*, collected July 11, 1938, on San Nicolas Island off Southern California by Professor Loye H. Miller of the University of California at Los Angeles. There were 56 parasites seen on one slide and 22 specimens on a second slide from the same bird. Type material is in the private slide collection of the authors.

This parasite is similar to *Sergentella hominis* Brumpt (Brumpt, 1927) in its spindle-like form and spiral lines but differs from it in its smaller, oval nucleus, knobbed extremities and much larger size. From the visible structure of *Spirogregarina fusiformis* in stained smears, it seems most closely related to certain developmental stages of haemogregarines. Because of its distinctive form, it is impossible to identify it with any known genus of the SPOROZOA.

MULTIPLE INFECTIONS

As is evident from a glance at Table 1 comparing number infected and total types of parasites indicated, many birds harbored more than one parasite. For those cases in which only one infected bird was found, the table indicates the types of parasites. The organisms occurring in mixed infections were *Trypanosoma* (T), *Haemoproteus* (H), *Plasmodium* (P), *Leucocytozoon* (L), intra-leucocytic parasite (I), and microfilarial worms (M). By using the letters indicated above, the complete figures for the birds with two or more genera of parasites is indicated as follows: THLM in 1 California spotted owl and 1 russet backed thrush; THL in 1 Bullock oriole, 5 black-headed grosbeaks, and 1 Gambel sparrow; TPL in 1 Anthony green heron; HLM in 1 common mallard and 1 Gambel sparrow; THP in 1 western white-winged dove; TLM in 1 evening grosbeak; TH in 2 hooded orioles, 2 Bullock orioles, 1 western tanager and 1 Gambel sparrow; TL in 2 American pintail, 1 Brewer black-bird and 1 evening grosbeak; HP in 1 hooded oriole and 2 common house finches; HL in 4 western mourning doves, 1 western tanager, 5 black-headed grosbeaks and 4 Gambel sparrows; HM in 1 hooded oriole and 2 Gambel sparrows; PL in 2 California jays, 1 western mockingbird and 2 common house finches; PI in 1 dwarf cowbird; and LM in 1 red-breasted merganser and 1 western robin.

It is interesting to note that the most common double infection was with *Haemoproteus* and *Leucocytozoon* (14 cases), whereas the commonest triple infection involved *Trypanosoma*, *Haemoproteus* and *Leucocytozoon* (7 cases).

Of 26 infected western mourning doves, 15 were infected with *Haemoproteus columbae*, 1 harbored *Haemoproteus sacharovi*, and 10 were infected with both species. Of 4 infected western white-winged doves, 3 harbored *Haemoproteus columbae*, and 1 was infected with *H. columbae* and *H. sacharovi*.

SUMMARY

In the present survey, 1,525 birds of 112 species and subspecies were examined. Three hundred fifty-seven individuals or 23.4 per cent, representing 54 species or 48.2 per cent, were infected. Trypanosomes were found in 42 birds of 22 species including the following new host records: Anthony green heron, American pintail, western white-winged dove, California spotted owl, eastern flicker, western flycatcher, russet-backed thrush, yellow warbler, Macgillivray warbler, pileolated warbler, hooded oriole, Bullock oriole, Brewer blackbird, dwarf cowbird, western tanager, black-headed grosbeak, evening grosbeak, California purple finch, willow goldfinch, and Gambel sparrow.

Haemoproteus was observed in 159 birds of 21 species including the following new host records: American pintail, San Quintín quail, Gambel quail, mountain quail, band-tailed pigeon, western white-winged dove, spotted screech owl, California spotted owl, russet-backed thrush, Audubon warbler, black-throated gray warbler, hooded oriole, Bullock oriole, western tanager, black-headed grosbeak, and San Clemente house finch.

Plasmodium was found in 71 birds of 11 species including the following new host records: Anthony green heron, western white-winged dove, hooded oriole, dwarf cowbird, California towhee and San Diego song sparrow.

Leucocytozoon was found in 123 birds of 33 species including the following new host records: Anthony green heron, cackling goose, white-fronted goose, redhead, sharp-shinned hawk, band-tailed pigeon, western mourning dove, Chinese spotted dove, California spotted owl, western mockingbird, California thrasher, western robin, hermit thrush, russet-backed thrush, orange-crowned warbler, Calaveras warbler, olive warbler, yellow warbler, Audubon warbler, Bullock oriole, Brewer blackbird, dwarf cowbird, western tanager, black-headed grosbeak, lazuli bunting, evening grosbeak, willow goldfinch and Gambel sparrow.

Hepatozoon was found in the following new hosts: 2 San Diego titmice, 1 pallid wren-tit, 1 California thrasher, 1 Audubon warbler, 1 black-headed grosbeak and 2 common house finches.

Intra-leucocytic parasites were found in 3 western mockingbirds, 1 English sparrow, 1 dwarf cowbird and 2 common house finches. The first three birds represent new host records.

Microfilarial worms were found in 13 birds of 10 species including the following new hosts: red-breasted merganser, California spotted owl, western robin, russet-backed thrush, pileolated warbler, hooded oriole, black-headed grosbeak, evening grosbeak and Gambel sparrow.

A new genus and species of parasite, *Spiroregarina fusiformis*, was reported from the rock wren.

No parasites were observed in 57 species represented by 98 birds. Mixed infections were found in 52 birds of 20 species, 38 individuals were infected with two genera of parasites, 14 with three. Two common house finches were infected with both *Plasmodium cathemerium* and *P. relictum*.

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FLAGELLATE PARASITES OF MOSQUITOES WITH SPECIAL REFERENCE TO *CRITHIDIA FASCICULATA* LÉGER, 1902*

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Since Ross' original discovery of flagellate parasites in the intestines of mosquitoes in 1898 numerous investigators have found parasites of this type in many parts of the world. As the earlier records are summarized by Novy, MacNeal, and Torrey (1907), Wenyon (1926), and Speer (1927) they will not be listed here in full but will be referred to only as they bear on questions of nomenclature and biology. The records made since Wenyon's and Speer's compilations are listed in Table 1.

TABLE 1.—Recent records of flagellate parasites found in mosquitoes

Date	Author	Locality	Host species	Per cent infected	Name of parasite
1926	Hindle & Patton	China	<i>Culex fatigans</i>		Undetermined
1926	Noguchi & Tilden	Georgia	<i>A. quadrimaculatus</i>		<i>H. culicidarum</i>
	" "	New Jersey	<i>Culex pipiens</i>		" "
1928	Shakhov	Russia	<i>A. maculipennis</i>	5 to 7	<i>C. fasciculata</i>
1930	Missiroli	Italy	<i>A. maculipennis</i>	2.3	<i>H. culicis</i> (= <i>C. fasciculata</i>)
1930	de Boissezon	France	<i>Culex pipiens</i>	(1 specimen)	<i>C. fasciculata</i>
1930	Taylor	Nigeria	<i>Lutzia tigrives</i>	(1 specimen)	Undetermined
1933	Feng	China	<i>A. jeyporensis</i>	3.3	<i>H. culicis</i> (= <i>C. fasciculata</i>)
			<i>A. hyrcanus</i>	12.5	<i>H. culicis</i> (= <i>C. fasciculata</i>)
			<i>A. sinensis</i>		"Flagellates including <i>C. fasciculata</i> "
1935	Barber & Rice	Macedonia	<i>A. maculipennis</i>		
1941	Ayroza & Coutinho	Brazil	<i>A. oswaldoi</i>	7.35	
			<i>A. triannulatus</i>	3.44	<i>H. pessoai</i>
			<i>davisi</i>		

(A. = *Anopheles* throughout)

NOMENCLATURE

The first technical name given to a mosquito flagellate was *Crithidia fasciculata* Léger, 1902. Léger described the species as small flagellates, 4 μ to 10 μ long, shaped like a barley corn, truncate at the anterior end and, according to one figure, with a funnel-like depression at the anterior end. In addition to these forms and connected to them by a series of intermediate stages he found more elongate forms (8 μ to 14 μ) which might have had an undulating membrane; "— sur l'un des côtés, le corps, plus aminci et à contour ondulé montre comme un rudiment de membrane ondulante." His casual sketches leave doubt as to whether an undulating membrane was actually seen or not.

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Novy, MacNeal, and Torrey (1907) made an extensive study of mosquito flagellates redescribing *C. fasciculata* and isolating it in pure culture. They discovered and isolated a second species, *Trypanosoma (Herpetomonas) culicis*, which was longer and provided with a short undulating membrane, and suggested that L  ger may have been dealing with a mixed infection.

Patton (1908), on the basis of his life history studies of insect flagellates, thoroughly confused the nomenclature of these forms. Assuming that all short truncate flagellates are "young" forms while long slender ones are "adults," a thesis which he fails to support by experimental evidence, he says that, even though L  ger may have seen two species, his *Crithidia fasciculata* can not be based on the short or young form but that the longer forms, presumably with the undulating membrane, must be accepted as typical of the species. He thought that Novy, MacNeal, and Torrey's *C. fasciculata*, lacking a membrane, should be called *Herpetomonas culicis*, while their *H. culicis*, having an undulating membrane, should be called a *Crithidia*. Patton (1909) says: "A reference to a recent paper by Novy, MacNeal, and Torrey will shew that by following L  ger's description of the genus they have named a true *Crithidia* a *Herpetomonas*." This is a curious interpretation of taxonomic rules as a "true *Crithidia*" should, presumably, be based on the original description.

Woodcock (1914), believing that L  ger had seen only one species, thought that *C. fasciculata* really had an undulating membrane. The organisms found by Novy, MacNeal and Torrey and by himself, and lacking a membrane should, he thought, receive a different name, *Leptomonas fasciculata*. Speer (1926) pointed out that the use of the same specific name in two closely related genera is contrary to the international code and renamed Novy's form *L. michiganensis*. Subsequent authors have all followed Patton's and Woodcock's lead in assuming that the type of the genus *Crithidia* had an undulating membrane.

Actually L  ger, in his original designation of *Crithidia fasciculata*, gave a distinctive and recognizable description of a short truncate membraneless flagellate which attaches in masses to the walls of the hind gut. The existence of such flagellates in mosquitoes has been confirmed, in the study of parasites from insects and from cultures, by many authors; most critically by Novy et al (1907), Fraenkel (1909, working with Novy's strain), Woodcock (1913), N  ller (1917), Schulz (1924), Shakhov (1928), Thomson (1930, Noguchi's strain), Lwoff and Lwoff (1931), and the present author. None of these workers found an undulating membrane in any stage. The present author has studied these parasites in naturally infected mosquitoes, in cultures, and in mosquitoes artificially infected with pure cultures. L  ger's reference to longer forms which may have had an undulating membrane appears, in his paper, after the description of the membraneless "barleycorn" type which was clearly the form meant to be called *Crithidia fasciculata*. If he actually saw an undulating membrane, a point that is not indisputably made, it is much more probable that he had a second species than that he had a species identical with the forms found and studied many times since, except for the possession of a membrane. His reference to intergrading forms can not be accepted as indicating the unity of these organisms as, while there may be an overlapping in size ranges, the presence or absence of a membrane is a sharp and definite character.

Novy, MacNeal and Torrey's description of *Herpetomonas culicis* shows that there is a flagellate in mosquitoes with a membrane and distinct from *Crithidia*

fasciculata in the host and in culture. Mezincesco (1908), Missiroli (1930) and the present author have confirmed this to the extent of finding two distinct types in mosquitoes; one short, truncate, membraneless, and attached to the walls of the hind gut and the other longer, provided with a membrane, and free in the mid gut. Ayroza and Coutinho (1941) made a careful study of *Herpetomonas pessoai* from the mid gut of anophelines which has not only stages with a short undulating membrane but trypanosome-like stages with a post-nuclear kinetoplast and a long undulating membrane.

The rediscovery of flagellates identical with Léger's *C. fasciculata* and lacking the membrane in all stages, the known existence of other species with the membrane in mosquitoes, and the discrediting of the life cycle of *Herpetomonas culicis* (= *C. fasciculata*) as described by Patton (which will be discussed below), remove the basis for stating that *Crithidia fasciculata* has an undulating membrane.

Thomson (1930) in describing Noguchi's strain of *H. culicidarum*, which, as pointed out by Nöller (1928) is identical with *C. fasciculata*, noted the funnel-like depression in the anterior end, which was suggested in one of Léger's sketches, and the whirling motion of the flagellum. Lwoff and Lwoff (1931), using Nöller's strain of *C. fasciculata*, pointed out these features and discovered 12 spiral lines on the surface which were stained by mitochondrial techniques. They erected the new subgenus *Strigomonas* in the genus *Leptomonas*, for this form but in view of the above arguments for stating that *Crithidia fasciculata* has no undulating membrane *Strigomonas* becomes a synonym of *Crithidia*.

We may, then, characterize *Crithidia fasciculata* as short flagellates rounded or pointed posteriorly, truncate and with a funnel-shaped depression anteriorly. Nucleus at or behind middle of cell. Kinetoplast slightly anterolateral from nucleus. Axoneme extending just under cell membrane to anterior end where it emerges excentrically. Cell membrane with about 12 spiral striae shown by mitochondrial technique. Organisms tend to attach themselves in clusters or to walls of host intestine. Parasites of hind gut of mosquitoes.

Members of the genus *Crithidia* differ from *Leptomonas* in the short anteriorly truncate shape, the funnel-like depression at the anterior end, the median or posteriorly situated kinetoplast, and the long axoneme emerging as the flagellum excentrically from the anterior end. They differ from members of the genus *Herpetomonas* in the same respects and in the complete absence of the undulating membrane.

It is with reluctance that the author proposes to discontinue the long established use of the name *Crithidia* as a generic name for trypanosomids with the short undulating membrane or as a term descriptive of such stages of members of the genera *Herpetomonas* and *Trypanosoma*, but as this use of the term appears to be based on a misconception of the type species and its description we have no choice.

LIST OF NAMES USED FOR MOSQUITO FLAGELLATES

Crithidia fasciculata Léger 1902

Synonyms: *Herpetomonas culicis* Novy, MacNeal and Torrey, 1907, as used by Patton and others

Leptomonas fasciculata Woodcock, 1914

Leptomonas michiganensis Speer, 1926

Herpetomonas culicidarum Noguchi and Tilden, 1926

Crithidia anophelis Missiroli, 1930

Leptomonas (Strigomonas) fasciculata Lwoff and Lwoff, 1931

Herpetomonas algeriense Sergent and Sergent, 1906

Herpetomonas culicis Novy, MacNeal and Torrey, 1907

Herpetomonas myzomyiae Drbholav, 1925 (insufficiently described)

Herpetomonas pessoai Ayroza and Coutinho, 1941

MATERIALS AND METHODS

Rearing mosquitoes: A colony of *Culex pipiens*, established from hibernating females among which the flagellate infections studied were found, has been kept through six generations. The species was determined by study of male genitalia and larval structure. The wild-caught females were fed raisins and oviposited without a blood meal. Neither these nor subsequent generations would bite human beings though offered many opportunities. The cage-reared females were fed on day-old mice and canaries as well as on soaked raisins. Larvae were reared in finger bowls and fed a variety of food including bread crumbs, dog biscuit, powdered milk, and yeast. Eighty-five adults and many larvae from the colony were examined as controls but none was found infected with protozoa.

A colony of *Aedes aegypti*, originally obtained from the Army Medical School, Washington, and reared according to the usual methods, was also used for experimental infections.

Dissection of mosquitoes: The entire intestine of the mosquito was drawn out as in examination of *Plasmodium* oöcysts and mounted with care to avoid cover-glass pressure. Infected intestines were studied in toto and then smeared for staining with Giemsa's stain.

Isolation of pure cultures: When culturing was to be attempted each mosquito was anesthetized, the legs and wings were cut off, and the body was dropped for two minutes in a dish of 0.6 per cent Hg Cl₂ to which a drop of tergitol had been added. After this bath it was washed successively in six tubes of sterile saline, the transfers being made with a flamed wire loop. The dissection and examination were done with sterile precautions, the needles being washed in alcohol and then sterile saline, and the slides and covers flamed. Infected intestines were teased in sterile broth and the suspension streaked on plates and planted in tubes.

Plates were made by adding 2 cc of human blood cells, left after plasma is drawn off, or 3 cc of rabbit blood to 10 cc of Difco nutrient agar adjusted to litmus neutrality. In tubes, N.N.N., Difco nutrient agar with or without blood, or neutral peptone broth with or without blood were used. The best growth was obtained on N.N.N. tubes to which 1 cc of peptone broth each was added but abundant growth occurred on all media used. All cultures were kept at room temperature.

OCCURRENCE AND ISOLATION

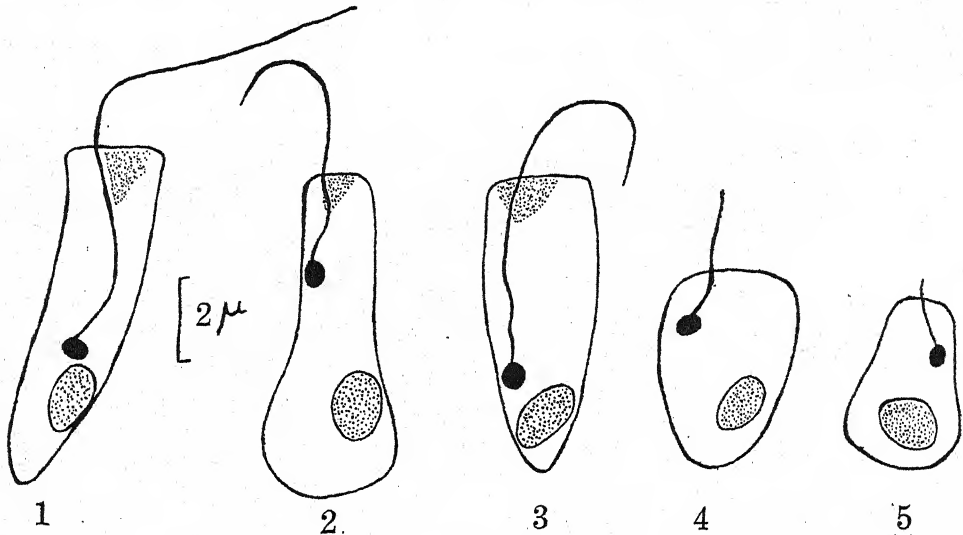
Among 18 *Culex pipiens* collected from a cave on the bank of the Mississippi in Saint Paul, Minn., in January 1942, 8 were found infected with flagellates. Two were infected with long slender forms which were free in the hind and mid guts. The difference between these and *Crithidia fasciculata* as seen in fresh mounts of the entire intestine, is striking. The organisms were studied in fresh and Giemsa-stained smears and identified as *Herpetomonas culicis* Novy, MacNeal and Torrey.

Six of the mosquitoes were infected with short flagellates attached in enormous numbers to the walls of the hind gut which were identified as *Crithidia fasciculata* Léger. Cultures were made and a number of strains persisted through several transplants, though heavily contaminated with bacteria. Attempts to isolate these strains failed and they died out.

On March 19, one specimen among 30 *Culex pipiens* from the same cave was found infected with *Crithidia*. The organisms were plated and planted in a tube of N.N.N. medium. The plates failed to show growth but a rich culture of flagellates mixed with yeast appeared in the tube. This mixed culture grew abundantly through five transplants. After a number of attempts pure colonies were obtained on blood agar plates after 6 days' incubation and the strain thus isolated has been maintained since.

MORPHOLOGY

Crithidia fasciculata in the active or nectomonad form is from 6μ to 8μ long and 2μ to 3μ wide. The shape, while variable, exhibits certain features which



FIGS. 1 to 5. *Crithidia fasciculata* as seen in Giemsa stained smears.

distinguish it from *Herpetomonas culicis* and other flagellates. The anterior end is truncate, sometimes with a very slight narrowing behind it so that the anterior tip is vase-shaped. There is a funnel-shaped depression in the anterior end. Usually the widest point is at about the middle of the length, and the posterior end tapers to a point that is slightly rounded off. Sometimes the posterior part is inflated and the anterior half cylindrical (Figs. 1-5).

In Giemsa-stained smears the nucleus is in the posterior half of the body. The kinetoplast is close in front of it, at or posterior to the middle of the cell and close to one side. From it the axoneme extends forward, near the surface to the anterior end where the flagellum emerges near one side (Fig. 6). There is no trace of an undulating membrane. This has been verified by dark-field study of living forms and careful study of living and stained slides. The author has confirmed the presence of about 12 spiral striae on the surface of the cell, first observed by Lwoff and Lwoff and shown by Altman's fuchsin technique.

The small rounded forms or haptomonads, which are attached to surfaces are $3\ \mu$ to $4\ \mu$ long and $2\ \mu$ to $4\ \mu$ wide. The nucleus, kinetoplast, and axoneme are seen in varying positions and the free flagellum is absent or very short. These forms are seen both in culture and in the mosquito. In culture they attach themselves to any surface; to the surface film of an uncovered drop, to the glass, or to an air bubble. Novy, MacNeal and Torrey called attention to their tendency to cluster around bubbles.

In the host all the flagellates are normally in the motionless haptomonad form and attached to the gut wall. When the intestine is very carefully drawn out and not pressed by the cover glass, that is seen to be the case. As the preparation is squeezed, more and more become free and swim about. If the intestine is broken so that the organisms escape into the saline, they all swim actively. This was noted by Ross in his pioneer work.

Patton (1907 and 1912) describes a life history for *Crithidia fasciculata* with rounded attached preflagellate stages in larvae, flagellate or adult stages in pupae and adults, and postflagellate stages in the rectum of the adult. The cycle was not worked out experimentally but by arranging the stages found in natural infections in an arbitrary sequence. There is no morphological difference between his pre-flagellate and postflagellate stages. His observations can be explained equally well on the basis of fortuitous variations in behavior as a result of differences in treatment during observation. In the experimental infections described below, no such sequence of stages could be found in larval and adult infections or in infections of varying duration. The majority of the organisms were always in the haptomonad or attached form until disturbed when they would become active. The only difference noted between infections of 50 minutes and 22 days' duration was in the number present.

BIOLOGY

The basic question regarding any haemoflagellate of blood sucking insects is whether it is a stage in the development of a parasite of vertebrates or is a parasite of the insect alone. While many workers have suggested, and a few have claimed experimental evidence, that flagellates of mosquitoes were stages in the development of parasites of birds or mammals, we have no acceptable proof that such is the case. The claims of Schaudinn (1904), the Sergents (1905), Laveran and Franchini (1914), and Fantham and Porter (1915) are discussed in extenso by Novy, MacNeal and Torrey and by Wenyon and need not be reconsidered here.

On the other hand observations by the Sergents (1906) and Patton (1912) provide circumstantial evidence that infection with certain species is acquired in the larval stage. The Sergents found natural infections of *Herpetomonas algeriensis* in *Culex* and *Aedes aegypti* which were reared from larvae in the laboratory. Patton found approximately 95 per cent of the larvae and pupae of *Culex fatigans* in a well at Madras infected with *H. culicis* (= *Crithidia fasciculata*) and concluded that the source of infection must have been the water. Hitherto no one has infected mosquitoes experimentally.

ATTEMPTS TO INFECT LARVAE

On April 16 a number of second and third stage *Aedes* and *Culex* larvae were dropped into a rich broth culture of *Crithidia* and after two minutes removed to

clean water. Organisms were seen in the gut of dissected larvae immediately afterward. Eighteen adults which emerged eight to twelve days later were uninfected.

On May 1 a number of fourth stage larvae of *Culex* were immersed in a broth culture for two minutes and then transferred to a bowl of clean water to which the culture was added. A larva dissected immediately after the immersion had numerous flagellates in the fore gut. One dissected after 50 minutes showed numerous flagellates in the mid gut, many being attached side by side to the gut wall as in the natural infections of adults. The next day a pupa which had formed in the meantime was found negative but two out of three larvae examined showed many organisms lined up along the walls of the mid and hind guts. A few living *Crithidia* were still in the water. Twelve adults which emerged in from four to six days were negative.

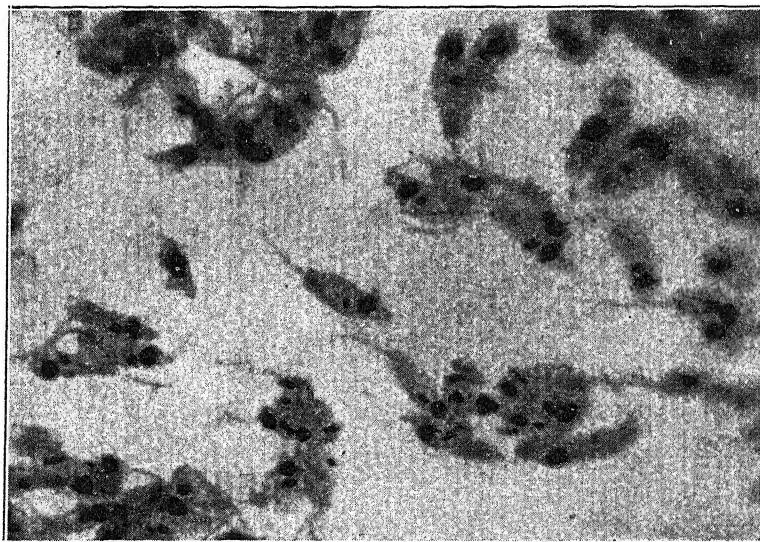


FIG. 6. Photomicrograph of *Crithidia fasciculata* stained with Giemsa's stain.

On May 11 and May 17 rich broth cultures of *Crithidia* were poured into a bowl of second stage *Culex* larvae. Fourteen adults which emerged between June 2 and 9 were negative.

The temporary infections of larvae indicate that the method of administering the protozoa was effective. The failure of the flagellates to remain into the adult stage can not be explained, though an abundant bacterial flora in the larvae may be detrimental to the protozoa. It is possible that with larvae reared under different conditions the parasites acquired in that stage will persist into the adult stage.

INFECTION OF ADULT MOSQUITOES

Adult *Culex pipiens* and *Aedes aegypti* can be readily infected with *Crithidia fasciculata* in the laboratory. Mosquitoes are put in lantern globes with netting tops and kept in a moist cabinet without food or water for 24 hours. Then a sterile cotton swab is dipped in a culture of *Crithidia* and is either offered to the mosquitoes one by one, if patience permits, or is simply left in the lantern globe until dry. A

drop of sugar solution on the swab appears to make the culture more acceptable. All of 15 mosquitoes which were known to have fed on the culture became infected. Of 38 mosquitoes exposed to the infection by having opportunity to feed on the culture (though no observations were made as to whether they actually drank or not), 20 became infected. Mosquitoes were examined as early as three days and as late as 22 days after infection. In the three-day infection parasites were abundant and in thick clusters in the hind gut but they did not cover the entire surface. In all infections examined after six days or more the flagellates were as abundant as in the natural infections. They cover the entire lining of the hind gut, rectal pouch, and rectal papillae like a carpet and the lumen is packed with clusters (Fig. 7). It is suggested that in laboratories where mosquito colonies are kept such demonstra-

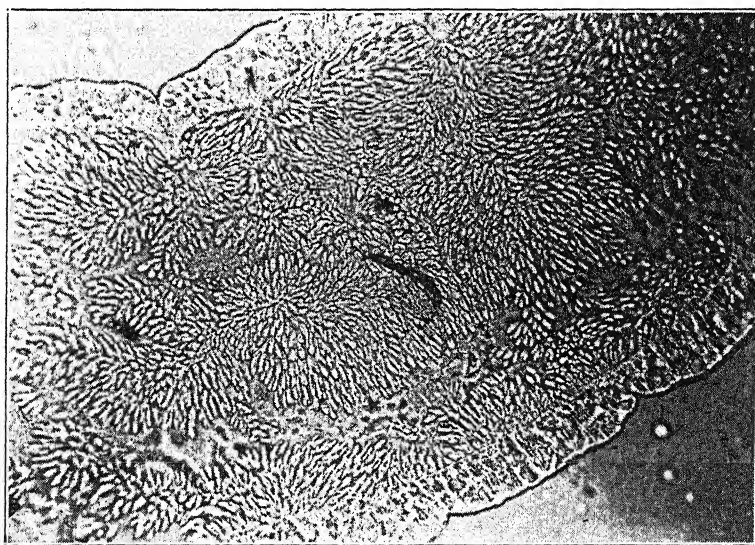


FIG. 7. Photomicrograph of fresh preparation of hind gut of mosquito infected with *Crithidia fasciculata*.

tions of infected mosquitoes are very easily prepared and are a striking supplement to laboratory work on insect born flagellates.

TRANSMISSION

A number of *Culex pipiens* which had had opportunity to feed on a *Crithidia* culture were caged in clean lantern globes with uninfected mosquitoes, which were marked by cutting off the end of the wing. This mark was easy to recognize at the end of the experiment.

In one globe three infected females were placed with six males and six females, all uninfected. Twenty days later the one of the originally exposed specimens still alive was found infected. Five males and five females of the test group were infected, while one of each sex was negative.

In another globe nine exposed males were caged with six uninfected males and six uninfected females. Twenty days later the insects remaining were examined. Five of the originally exposed males were infected and three were negative. Of the

test males two were negative and two positive and of the females four were positive and two negative.

This experiment shows that in the limited confines of a lantern globe the infection is readily passed from one mosquito to another. This presumably occurs through the ingestion of feces as numerous droplets are found on the glass surface but the exact mechanism of transfer was not determined. There is no evidence and little probability that fecal contamination in nature, unless mosquitoes are breeding in a small space, is a factor in the natural dissemination of the parasite.

SUMMARY

1. *Crithidia fasciculata* Léger, 1902, type of the genus *Crithidia*, has no undulating membrane.
2. Patton's life cycle of *Herpetomonas culicis* (= *C. fasciculata*) involving pre-flagellate, flagellate, and postflagellate stages is erroneous.
3. The parasites are normally attached in the small rounded form to the gut lining but become free when disturbed.
4. Transitory infections were produced in larval mosquitoes by feeding cultures of *Crithidia* but the organisms did not persist into the adult stage.
5. Adult mosquitoes are easily infected by feeding them cultures.
6. *Crithidia fasciculata* is transferred from one mosquito to another when the insects are confined in lantern globes.

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FOOD HABITS AND INTENSITY OF COCCIDIAN INFECTION IN NATIVE VALLEY QUAIL IN CALIFORNIA¹

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Examination of fecal samples from large series of California quail (*Lophortyx californica*), taken in various areas in California during 1941, showed a high incidence of coccidian infection. Herman and Jankiewicz (1942) have demonstrated that the infection reduces to a minimum in the absence of material contaminated with ripe coccidian oöcysts. Herman, Jankiewicz and Saarni (1942) showed further that, under natural conditions, the intensity of infection in individual quail tends to fluctuate, probably decreasing as the parasites are eliminated and increasing with the repeated ingestion of oöcysts.

A possible relationship between the intensity of coccidian infection and food habits was observed from data on fecal samples of quail from the San Joaquin Experimental Range, Madera County, California.

The type of food consumed by these birds varies according to the season. They subsist primarily on seeds during the dry summer and early fall, but after the first rains the diet becomes predominantly green leafy material, as demonstrated by Glading, Biswell and Smith (1940) from the analysis of quail stomachs taken on the experimental range.

Data for the present study are based on the examination of 342 fecal samples procured from April 1941, through February 1942. The procedure followed has been described by Herman, Jankiewicz and Saarni. Intensity was computed by the number of oöcysts present in a single drop of surface film of the concentrated zinc sulfate centrifuged material (1-10, 1+; 11-50, 2+; 51-100, 3+; 101-500, 4+; 501-1000, 5+; over 1000, 6+). Tabulations were made by individual samples, no consideration being given to individual birds. In computing the data presented in section C of the graph, negatives were considered as 0, each 1+ infection as 1, etc. The average (points plotted) was obtained by dividing the sum of the infections for each month by the number of samples examined. Section A of the graph presents the actual inches of rainfall during the period of the study. Section B pictures the probable food consumption (for the period March 1941, through February 1942) interpolated from 1937 data of relation of rainfall to per cent of composition of food consumed (after Glading, Biswell and Smith, 1940).

Becker and Waters (1939) reported that alfalfa contains liberal amounts of the hypothetical materials for promoting coccidium growth as compared with grains,

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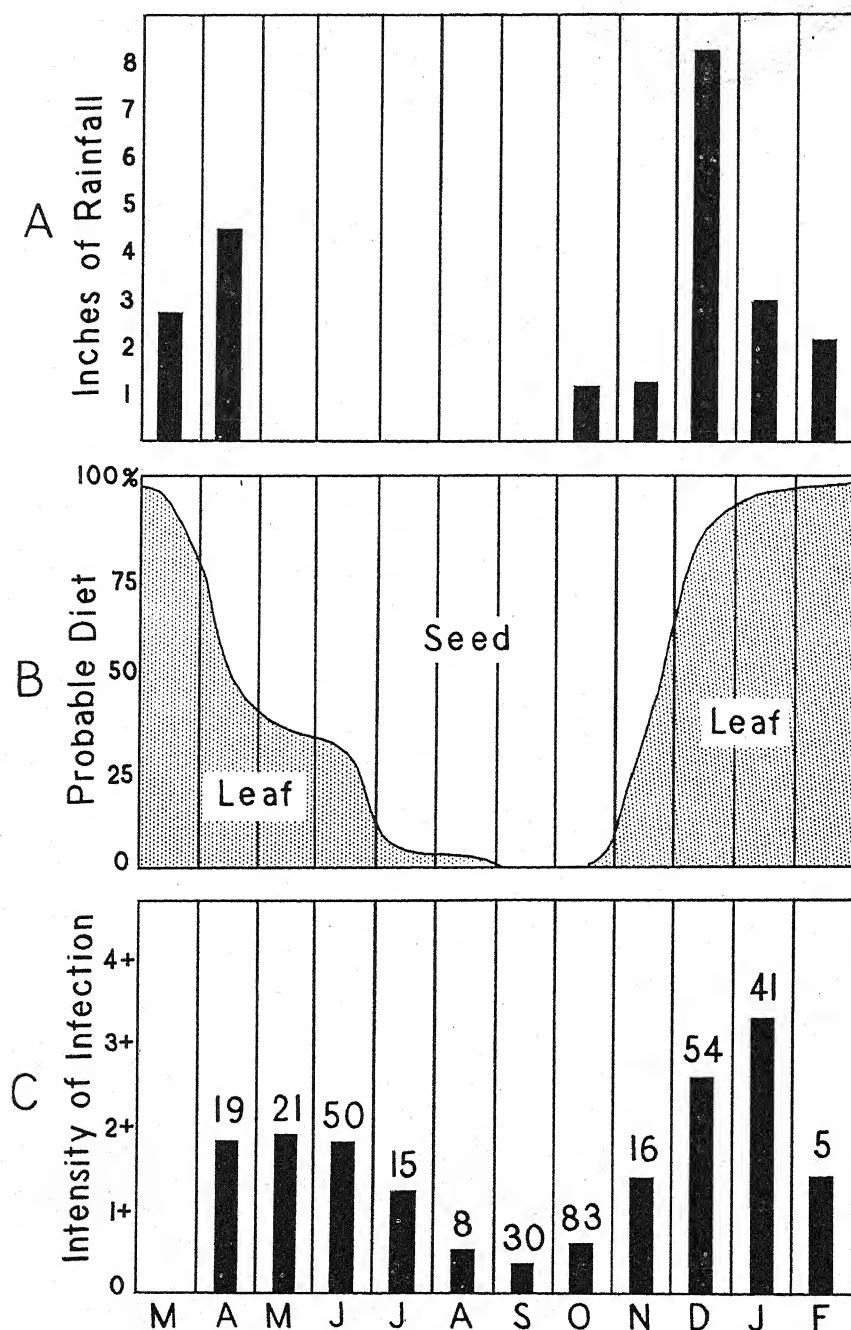


FIG. 1. Graphs indicating correlation of food habits with intensity of coccidian infection. A, inches of rainfall March 1941, through February 1942; B, probable food consumption (for period March 1941, through February 1942) interpolated from 1937 data of relation of rainfall to per cent of composition of food consumed (after Glading, Biswell and Smith, 1940); C, average intensity of coccidian infection, the figures above each bar indicating the number of fecal samples examined.

and we conjecture that other green foods may act similarly. The scope of this study has been limited by the relatively small number of samples examined and by its duration of only one year; also, very few samples were procured during some of the months. However, upon examination of the tabulated data as presented in the graphs, there seems to be a correlation between the consumption of green feed and the intensity of coccidian infection in quail living in a state of nature.

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SOME OBSERVATIONS ON SPLEEN VOLUME IN DOMESTIC FOWLS IN THE COURSE OF *PLASMODIUM GALLINACEUM* STUDIES¹

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The lymphoid-macrophage system is a most important factor in the resistance of an animal to malaria. The spleen is a large element in this system and must be considered in any account of the physical or chemical bases of protozoan immunity. (For reviews and references see Taliaferro, 1929 and 1941; Culbertson, 1941; and Hewitt, 1940. Perla and Marmorston, 1935, discuss the human spleen in relation to resistance.)

This paper reports some observations on spleen volume in domestic fowls made in the course of studies on *Plasmodium gallinaceum* infections.

SPLEEN VOLUME IN NONMALARIOUS FOWLS

In Table 1 are given data regarding spleen volume in 17 nonmalarious, and probably normal, domestic fowls. The fowls averaged about 10 weeks in age when purchased, but it was not possible to ascertain exact figures. It will be seen, in Table 1, that the average weight of the nonmalarious fowls was 634 grams and that the average spleen volume was 0.88 cc. This volume was obtained by noting the displacement of the spleen in a measuring cylinder. The spleen was carefully removed while the fowl was anaesthetized with chloroform.

METHOD OF ANALYSIS

Obviously spleen volume is greater in heavier fowls, and it was necessary to find an expression of volume which would take into account the weight of the fowl. This was done by computing the correlation and fitting a regression line to the data for the 17 normal fowls given in Table 1. The correlation coefficient was found to be

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TABLE 1.—Non-malarious untreated fowls: observed spleen volume and that computed from the regression of spleen volume on fowl weight*

Fowl serial number	Weight in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
		Observed	Expected	
255	724	1.2	1.08	+ 0.12
256	625	0.9	0.86	+ 0.04
262	862	2.0	1.38	+ 0.62
263	519	0.75	0.63	+ 0.12
264	663	0.75	0.95	- 0.20
275	560	0.6	0.72	- 0.12
276	553	0.5	0.71	- 0.21
277	725	0.8	1.08	- 0.28
278	555	0.5	0.71	- 0.21
279	606	1.2	0.82	+ 0.38
280	493	0.9	0.58	+ 0.32
298	577	0.75	0.76	- 0.01
299	630	1.5	0.87	+ 0.63
300	610	0.5	0.83	- 0.33
301	655	0.6	0.93	- 0.33
302	605	0.8	0.82	- 0.02
303	815	0.75	1.27	- 0.52
Total	10,777	15.00	15.00	0.00
Average per-fowl	634	0.88	0.88	0.00 \pm 0.08

* Regression equation for computing theoretical values: Spleen volume in cc = 0.002165 (fowl weight in grams) - 0.490129 .

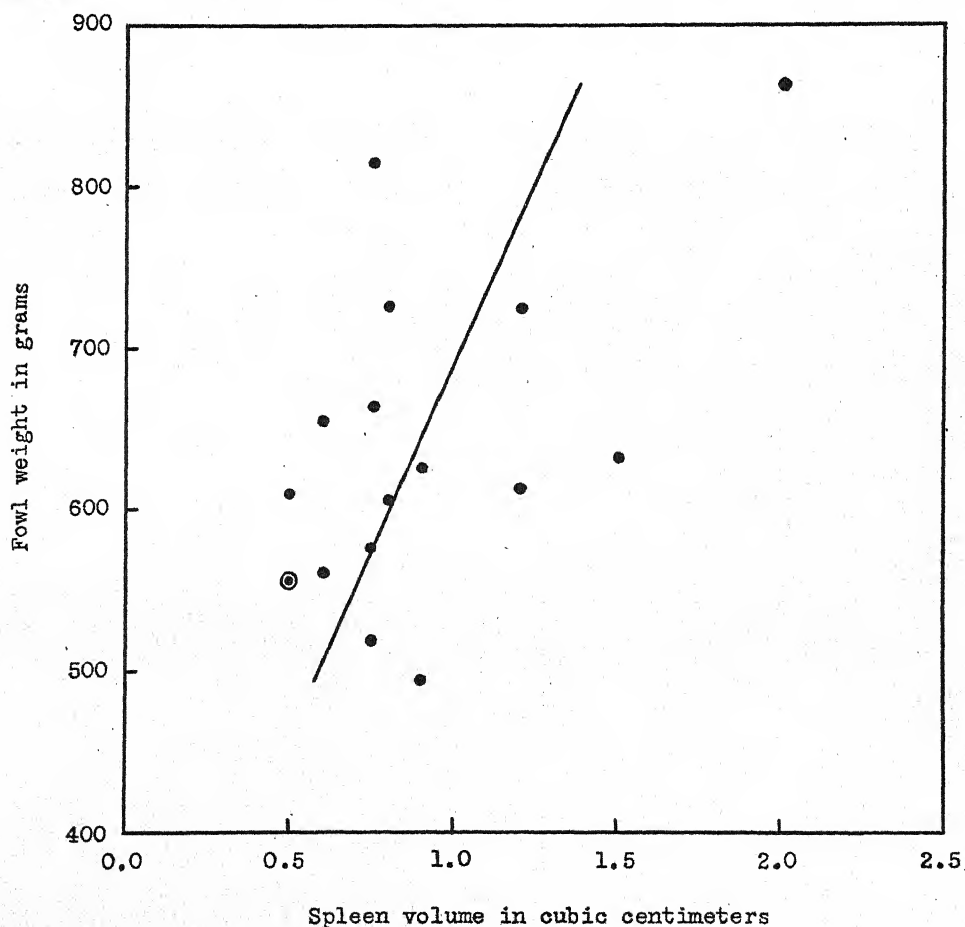


FIG. 1. Diagram showing the regression of spleen volume on fowl weight for a group of 17 normal birds.

+ 0.500, and the regression of spleen volume on weight is expressed by the following equation:

Spleen volume in cc = 0.002165 (fowl weight in gms) - 0.490129 . The correlation diagram with the fitted line for 17 normal fowls is shown in Fig. 1.

It is apparent that the observed values of weight and volume deviate considerably from the fitted line, particularly those for the heavier birds. The amount of deviation may be expressed in the form of a standard error² which will be smaller than one based on deviations from the average of observed spleen volumes, since the increase in volume per increase in weight has been taken into consideration. In Table 1 the sum of the deviations shown is zero with a standard error of ± 0.08 cc.

If the equation may be considered an adequate expression of the normal relationship between spleen volume and body weight it may be used to compute the expected spleen volumes for the fowls in each treated group. These expected values may then be compared with the observed spleen volumes following treatment. Each expected value will correspond to a point on the line shown in Fig. 1, and the amount and direction of observed from computed values may be used as a measure of the effect of treatment.

Fisher, 1938, gives a *t* test which may be used for determining the statistical significance of the differences between expected and observed values. The tables which follow contain the individual differences and their mean with standard error³ to which the test was applied. While the standard error does not take into account all possible variation of normal versus experimental spleen volumes, the differences in most instances are so great that the test is satisfactory for the purpose of this paper.

EFFECT OF INJECTIONS OF SHEEP SERUM

In Table 2 are included data for 17 fowls which received injections of normal sheep serum intravenously, subcutaneously, or intraperitoneally. Fowls 247 and 248 received intravenous injections of 1 cc sheep serum on March 31, April 7, and April 14, 1941. The spleens were removed on April 15. Fowls 259 to 261 received 0.25 cc sheep serum subcutaneously over the breast muscle daily from April 15 to 21, 1941. Spleens were removed on May 3. Fowls 281 to 286 received similar injections every second day from May 3 to 15, 1941. Spleens were removed on May 17.

The average weight of these fowls was 636 grams and the average expected spleen volume was 0.89 cc, about the same as in the untreated group. But the average observed spleen volume was 1.68 cc, nearly double that expected. The difference of $+0.79 \pm 0.12$ cc is significant. Evidently there was an increase in splenic volume following these injections.

EFFECT OF VACCINATION WITH INACTIVATED SPOROZOITES

In Table 3 are given data for seven fowls which received sporozoite vaccination. Each fowl received sporozoites from 200 mosquitoes injected intravenously in five equal doses between December 23 and January 20. In the case of fowls D 43, 45, and 54, salivary glands were dissected in saline and exposed to ultraviolet radiation for 30 minutes before injection. In fowls 47, 49, 51 and 52, thoraces from infective

² Standard error = $\left(\frac{\sum (\text{differences observed vs. expected volumes})^2}{n(n-2)} \right)^{1/2}$ in which *n* is equal to the number of fowls in the group.

³ Standard error computed as above with $n(n-1)$ as the denominator.

TABLE 2.—*Spleen volume of non-malarious fowls after injections of sheep serum*

Fowl serial number	Injections			Weight in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
	No.	Amount total cc	Route		Observed	Expected	
247	3	3.0	i/v	723	2.5	1.08	+ 1.42
248	3	3.0	i/v	751	1.75	1.14	+ 0.61
259	7	1.75	s/c	504	1.5	0.60	+ 0.90
260	7	1.75	s/c	575	1.9	0.75	+ 1.15
261	7	1.75	s/c	670	1.5	0.96	+ 0.54
265	7	7.0	i/p	587	1.7	0.78	+ 0.92
266	7	7.0	i/p	449	1.5	0.48	+ 1.02
267	7	7.0	i/p	583	2.25	0.77	+ 1.48
268	7	7.0	i/p	550	1.5	0.70	+ 0.80
269	7	7.0	i/p	773	1.9	1.18	+ 0.72
270	7	7.0	i/p	676	1.6	0.97	+ 0.63
281	7	7.0	i/p	742	2.9	1.12	+ 1.78
282	7	7.0	i/p	570	1.0	0.74	+ 0.26
283	7	7.0	i/p	729	1.2	1.09	+ 0.11
284	7	7.0	i/p	682	1.75	0.99	+ 0.76
285	7	7.0	i/p	612	1.0	0.83	+ 0.17
286	7	7.0	i/p	643	1.1	0.90	+ 0.20
Totals				10819	28.55	15.08	+ 13.47
Average per fowl				636	1.68	0.89	+ 0.79 \pm 0.12

mosquitoes were ground and dried and a saline suspension was injected. (For details regarding technique and significance of these procedures see Mulligan et al, 1941; Russell et al, 1942; Russell and Mohan, 1942.) The fowls were sacrificed on the day after receiving the last injection of vaccine.

The average weight of these seven fowls was 631 grams, and the expected average spleen volume was 0.88 cc, the same as that observed in normal fowls in Table 1. The average observed value, however, was 1.53 cc and the difference between expected and observed values was $+0.65 \pm 0.18$ cc and significant.

It should be noted that the observed spleen volumes in Tables 2 and 3 are all in excess of the expected values. This is a further indication that the spleens of these birds were abnormally large. If the deviations were distributed both below and above the expected values one could not be so sure of this.

EFFECT OF MALARIA

In Tables 4 and 5 data are given for spleens removed from fowls suffering from chronic malaria. The eight fowls reported in Table 4 were infected by blood inocu-

TABLE 3.—*Spleen volume of non-malarious fowls after vaccinations with inactivated sporozoites*

Fowl serial number	Method of inactivating sporozoites	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
			Observed	Expected	
D 43	u/v light	649	1.0	0.91	+ 0.09
D 45	u/v light	620	2.2	0.85	+ 1.35
D 54	u/v light	663	2.2	0.95	+ 1.25
Totals		1932	5.4	2.71	+ 2.69
Average per fowl		644	1.80	0.90	+ 0.90
D 47	Drying and grinding	654	1.2	0.93	+ 0.27
D 49	Drying and grinding	712	1.8	1.05	+ 0.75
D 51	Drying and grinding	445	0.8	0.47	+ 0.33
D 52	Drying and grinding	675	1.5	0.97	+ 0.53
Totals		2486	5.3	3.42	+ 1.88
Average per fowl		622	1.33	0.86	+ 0.47
Grand totals		4418	10.7	6.13	+ 4.57
Average per fowl		631	1.53	0.88	+ 0.65 \pm 0.18

TABLE 4.—*Spleen volume of untreated chronic malarious fowls infected by blood inoculations*

Fowl serial number	Duration of infection days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
223	38	++++	609	2.8	0.83	+ 1.97
226	38	++++	591	1.5	0.79	+ 0.71
227	38	++++	760	4.1	1.16	+ 2.94
228	38	++++	657	2.8	0.93	+ 1.87
230	38	++++	639	3.0	0.89	+ 2.11
254	114	++	854	1.4	1.36	+ 0.04
258	38	++	625	1.8	0.86	+ 0.94
305	33	+	656	1.8	0.93	+ 0.87
Totals	375	5391	19.2	7.75	+ 11.45
Averages	47	674	2.40	0.97	+ 1.43 ± 0.33

NOTES: The plus signs follow Beckman's (1941) method of classification. As used by us they have approximately the following meaning:

- + Less than 1 per cent of cells infected
- ++ One to 4 per cent of cells infected
- +++ Five to 8 per cent of cells infected
- ++++ Nine to 12 per cent of cells infected
- ++++ More than 12 per cent of cells infected

lation and the 26 in Table 5 by mosquito-borne infection. The average weight of these 34 fowls was 657 grams when sacrificed, a little more than that of newly purchased birds since they had been well fed in the laboratory for several weeks. The observed average spleen volume of the 34 fowls was 2.28 cc. The expected average spleen volume was 0.93 cc, giving a difference of $+1.35 \pm 0.16$ cc, which is highly significant.

The difference in spleen volume between fowls infected by needle and by mosquito inoculation is not significant, either when comparison is made of the average observed or expected values. The mean duration of infection in the first group was 47 days, and in the second 39 days, from date of inoculation to that of removal of spleen.

TABLE 5.—*Spleen volume of untreated chronic malarious fowls infected by mosquitoes*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
195	80	++	899	1.2	1.46	- 0.26
197	78	+	892	1.9	1.44	+ 0.46
219	55	+	696	1.5	1.02	+ 0.48
220	55	+	658	1.8	0.93	+ 0.87
320	24	++++	428	2.2	0.44	+ 1.76
321	23	++++	654	2.8	0.93	+ 1.87
323	23	++++	1007	3.8	1.69	+ 2.11
325	23	++++	564	2.7	0.73	+ 1.97
326	23	++++	507	1.8	0.61	+ 1.19
327	23	+++	686	4.0	1.00	+ 3.00
328	23	++++	433	2.7	0.45	+ 2.25
395	55	++++	486	1.0	0.56	+ 0.44
398	54	++++	484	1.2	0.56	+ 0.64
400	73	++++	671	1.8	0.96	+ 0.84
413	41	++++	629	2.0	0.87	+ 1.13
414	41	++++	657	1.5	0.93	+ 0.57
415	41	++++	612	4.0	0.83	+ 3.17
416	40	++++	588	2.0	0.78	+ 1.22
418	30	++++	597	1.4	0.80	+ 0.60
419	40	++++	461	1.8	0.51	+ 1.29
420	40	++++	702	1.9	1.03	+ 0.87
421	39	++++	453	0.9	0.49	+ 0.41
423	39	+++	736	3.0	1.10	+ 1.90
A 94	21	++	959	5.0	1.59	+ 3.41
A 97	21	+++	743	2.4	1.12	+ 1.28
A 98	21	++	739	2.0	1.11	+ 0.89
Total	1026	16941	58.3	23.94	+ 34.36
Average	39	652	2.24	0.92	+ 1.32 ± 0.18

That the spleen volume per fowl weight decreases as the infection becomes more chronic in these fowls is seen in the following arrangement of data from Tables 4 and 5.

Duration of infection in days	Number of fowls	Average weight in gms	Average spleen volume in cc		Difference observed vs. expected
			Observed	Expected	
20-29	10	672	2.94	0.97	+ 1.97 \pm 0.25
30-39	10	632	2.31	0.88	+ 1.43 \pm 0.26
40-49	6	608	2.20	0.83	+ 1.38 \pm 0.37
50 or more	8	705	1.48	1.04	+ 0.44 \pm 0.14

The excess of observed over expected spleen volume decreased as the duration of infection increased. The amount of the excess was significant for each group.

EFFECT OF SPOROZOITE VACCINATION WITH SUBSEQUENT MALARIA INFECTION

In Tables 6 and 7 are reported data regarding spleen volume in 14 fowls that

TABLE 6.—*Spleen volume of vaccinated chronic malarious fowls infected by blood inoculation*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
203	37	++++	705	2.0	1.04	+ 0.96
204	37	++++	756	4.0	1.15	+ 2.85
208	37	++++	710	2.75	1.05	+ 1.70
210	37	++++	1035	3.0	1.75	+ 1.25
211	37	++++	750	3.9	1.13	+ 2.77
Totals	185	3956	15.65	6.12	+ 9.53
Average	37	791	3.13	1.22	+ 1.91 \pm 0.39

TABLE 7.—*Spleen volume of vaccinated chronic malarious fowls infected by mosquitoes*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
165	83	++++	1317	2.0	2.36	- 0.36
171	83	++++	1036	2.0	1.75	+ 0.25
176	53	+++	947	2.1	1.56	+ 0.54
177	53	+++	768	1.75	1.17	+ 0.58
185	61	+++	869	2.2	1.39	+ 0.81
A 7	24	+	698	3.4	1.02	+ 2.38
A 11	24	+	734	2.2	1.10	+ 1.10
A 13	24	+	579	0.9	0.76	+ 0.14
A 22	24	+	679	3.1	0.98	+ 2.12
Totals	429	7627	19.65	12.09	+ 7.56
Averages	48	847	2.18	1.34	+ 0.84 \pm 0.30

were given sporozoite vaccination and subsequently infected with malaria. Those included in Table 6 were infected by blood inoculation, and all had heavy infections. Those shown in Table 7 were infected by mosquito-borne sporozoites, and all had light infections. (For description of vaccination technique and other details regarding fowls included in Table 6 see Russell et al, 1942; for fowls 165 to 185 in Table 7 see Mulligan et al, 1941; for fowls A 7 to A 22 see Russell and Mohan, 1942.)

In the trophozoite inoculated group (Table 6) the difference between average observed and expected spleen volumes was + 1.91 \pm 0.39 cc. In the sporozoite

infected group (Table 7) the difference was $+ 0.84 \pm 0.30$ cc. In both groups the observed spleens were significantly larger than they presumably would have been if normal.

EFFECT OF SERUM INJECTIONS WITH SUBSEQUENT MALARIA INFECTION

In Table 8 are data regarding four fowls which were infected after receiving

TABLE 8.—*Spleen volume of chronic malarious fowls, treated with serum, infected by mosquitoes*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowls in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
359	61	++++	504	1.1	0.60	+ 0.50
360	61	++	1040	2.5	1.76	+ 0.74
368	61	++++	431	0.9	0.44	+ 0.46
A 91	21	++	586	3.8	0.78	+ 3.02
Totals	204	2561	8.3	3.58	+ 4.72
Average	51	640.3	2.08	0.90	+ 1.18 \pm 0.62

serum injections. Fowls 359, 360, and 368 each received 1 cc of sheep serum daily from June 23 to 30, 1941, intraperitoneally. They were bitten by two infective mosquitoes on July 1. The infection ran for 61 days before the spleens were removed. Fowls 359 and 368 had severe infections, but in fowl 360 the infection was mild. Fowl A91 received daily injections of 1 cc of chronic fowl serum intraperitoneally from September 15 to 20. It was bitten by two infective mosquitoes on September 17.

The excess in observed spleen volume over that expected was small among the fowls in this group, with the exception of fowl A91. The long duration of the disease in the others may have been the cause of this.

EFFECT OF COMBINED SPOROZOITE VACCINATION AND SERUM INJECTIONS WITH SUBSEQUENT MALARIA INFECTION

In Table 9 data for 17 fowls are shown, each of which had both sporozoite vac-

TABLE 9.—*Spleen volume of chronic malarious fowls, vaccinated and treated with serum, infected by mosquitoes*

Fowl serial number	Duration of infection in days	Peak of infection	Weight of fowl in grams	Volume of spleen in cubic centimeters		Difference observed vs. expected value
				Observed	Expected	
233	23	++++	875	3.7	1.40	+ 2.30
234	23	++++	898	5.0	1.45	+ 3.55
235	23	++++	912	5.5	1.48	+ 4.02
236	22	++++	660	4.3	0.94	+ 3.36
237	22	++++	924	3.5	1.51	+ 1.99
238	22	++++	719	3.2	1.07	+ 2.13
239	30	++++	679	3.0	0.98	+ 2.02
241	22	++++	691	3.7	1.01	+ 2.69
272	22	++++	677	3.2	0.98	+ 2.22
357	60	++++	747	3.7	1.13	+ 2.57
393	42	++++	692	3.1	1.01	+ 2.09
401	40	++++	914	3.0	1.49	+ 1.51
A 46	21	+	890	3.5	1.44	+ 2.06
A 48	21	+	758	3.6	1.15	+ 2.45
A 51	21	+	793	3.0	1.23	+ 1.77
A 53	21	+	981	4.8	1.63	+ 3.17
A 60	21	+	1021	4.5	1.72	+ 2.78
Totals	456	13831	64.3	21.62	+ 42.68
Averages	27	814	3.78	1.27	+ 2.51 \pm 0.16

cination and serum injection (see Russell and Mohan, 1942). Fowls 233 to 272 were each vaccinated five times between April 17 and May 12. They also received 1 cc of sheep serum intraperitoneally daily from May 10 to 16. They were infected by two mosquitoes on May 19. Fowls 357 to 401 were vaccinated six times between June 22 and July 14, 1941, and each received 1 cc chronic fowl serum intraperitoneally daily from July 14 to 18. They were infected by two mosquitoes on July 20. Fowls A 46 to 60 were vaccinated five times between August 29 and September 13. They also received 1 cc chronic fowl serum daily by intraperitoneal injection from September 15 to 20. They were infected by two mosquitoes on September 17.

The excess of observed over expected spleen volume was greatest in this group of fowls. The average difference was $+2.51 \pm 0.16$ cc and is highly significant.

DISCUSSION

Table 10 summarizes the results of the observations made on these 110 fowls.

TABLE 10.—*Results of experiments designed to show the effect of various types of treatment upon the average spleen volume of fowls*

Table number	Description of fowl group	Number of fowls	Average weight in grams	Average spleen volume in cubic centimeters		Difference observed vs. expected with standard error
				Observed	Expected	
1	Nonmalarious, untreated	17	634	0.88	0.88	0.00 ± 0.08
2	Nonmalarious, serum treated	17	636	1.68	0.89	0.79 ± 0.12
3	Nonmalarious, vaccinated	7	631	1.53	0.88	0.65 ± 0.18
4	Chronic-malarious, blood inoculated	8	674	2.40	0.97	1.43 ± 0.11
5	Chronic-malarious, mosquito infected	26	652	2.24	0.92	1.32 ± 0.18
6	Chronic-malarious, vaccinated, blood-inoculated	5	791	3.13	1.22	1.91 ± 0.39
7	Chronic-malarious, vaccinated, mosquito infected	9	847	2.18	1.34	0.84 ± 0.30
8	Chronic-malarious, serum treated, mosquito infected	4	640	2.08	0.90	1.18 ± 0.62
9	Chronic-malarious, vaccinated and serum treated, mosquito infected	17	814	3.78	1.27	2.51 ± 0.16

The spleen in 17 normal fowls about 10 weeks in age averaged 0.88 cc in volume. It was possible to compute the regression of spleen volume on body weight for these normal fowls, by means of which the expected spleen volume in terms of weight of fowl could be computed for each bird in the experimental groups. This was done, and comparisons were made between observed and theoretical spleen volumes so that the effect of treatment could be evaluated. The following observations may be made:

Vaccination with inactivated sporozoites, whether the vaccine is prepared by exposure to ultraviolet radiation or by grinding and drying, increases the volume of the spleen in nonmalarious fowls.

The injection of serum, whether from normal sheep or from fowls chronically infected with the homologous plasmodium, causes the spleen to increase in volume. Sections from such spleens showed chiefly an increase in lymphoid tissue with somewhat enlarged sinuses engorged with lymphoid cells.

Manwell and Goldstein, 1940, observed this effect of serum upon the spleens of nonmalarious canaries. They found that injections of normal serum or even of physiological saline produced a splenic enlargement comparable to that produced by immune serum.

The spleen volume is increased by malaria infection (Tables 4 and 5) and there is no significant difference between blood-inoculated and mosquito-infected groups. Such enlargement has, of course, long been known to occur. It was interesting that the volume decreased as the chronic infection progressed.

The largest increase in volume was seen in the group of 17 chronically malarious fowls which had had both vaccination and serum treatment in addition to mosquito-borne malaria infection (Table 9).

SUMMARY

This paper presents data showing the spleen volume of normal fowls and the increased volume caused by chronic malaria, by serum treatment, by sporozoite vaccination, and by chronic malaria following sporozoite vaccination with and without serum treatment.

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OBSERVATIONS ON THE SEGMENTAL ANATOMY OF THE TAPE-
WORM, *MESOCESTOIDES VARIABILIS* MUELLER, 1928,
FROM THE OPOSSUM

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Recently one of us (J.W.W.) obtained several incomplete specimens of a tapeworm from the small intestine of the opossum, *Didelphis virginiana* Kerr, from Mississippi. Unfortunately only incomplete strobilae were obtained and in no case was a scolex recovered. Hence no measurements on the total length of single specimens can be made nor can we determine the total number of proglottides in the strobila of the individual. However a sufficient number of both mature and gravid proglottides was obtained for making whole mounts and sections. A study of these segments from the same chain and from similar chains in the collection has convinced us all of our material belongs to the same species of tapeworm.

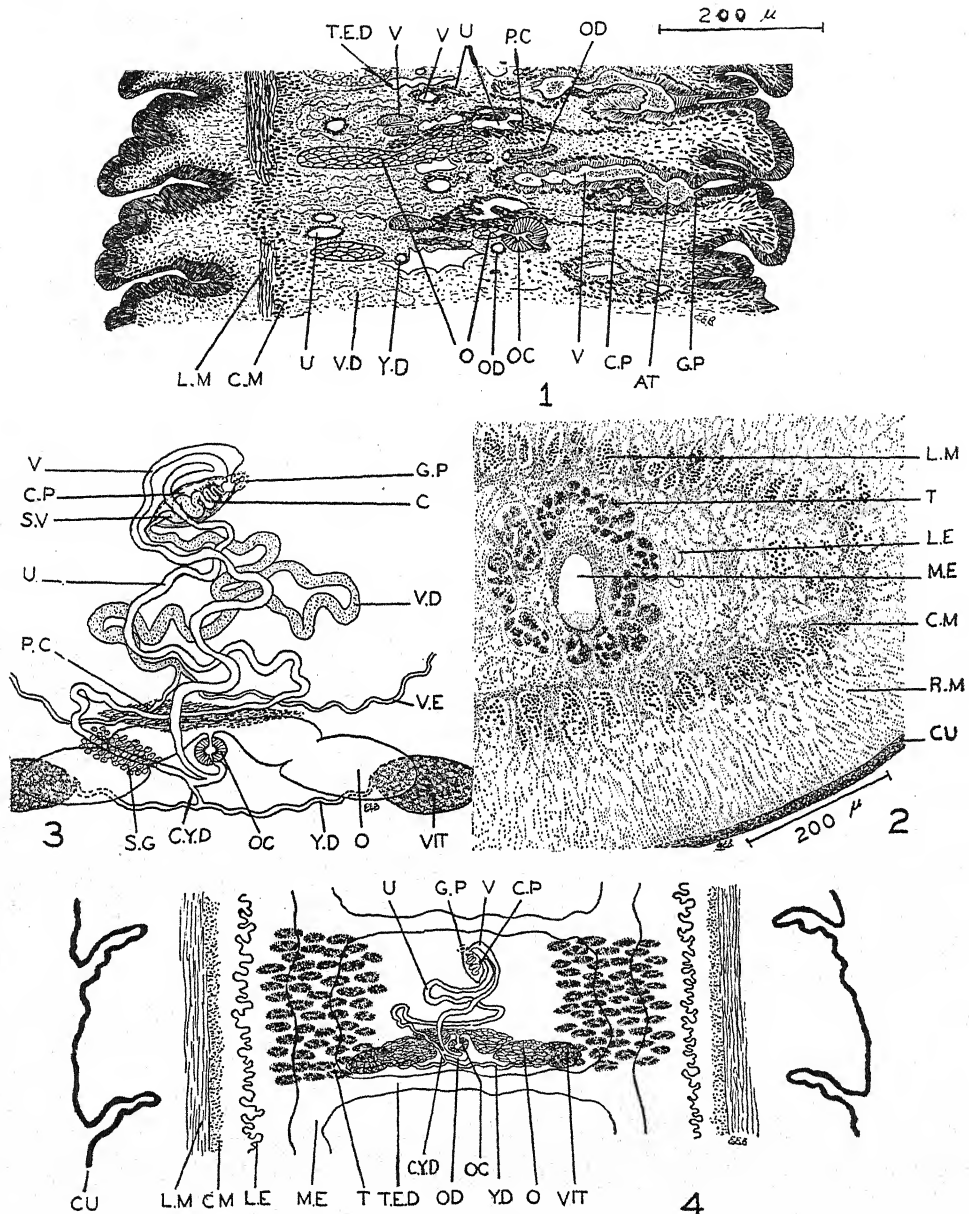
Although our material came from the opossum, the size of the mature and gravid proglottides, the nature and arrangement of the musculature within the segment, the presence of rather large calcareous corpuscles and the nature of the egg-capsule of the fully gravid segment definitely determine the species to be identical with *Mesocestoides variabilis* Mueller, 1928.

Since no complete strobilae were obtained we are permitted only to give a description of the anatomy of the segment. Such a detailed description seems to be timely since in our opinion no paper familiar to us on the group of tapeworms here considered adequately describes the connections made by the various ducts that compose the reproductive system. Therefore in the present paper we propose to give a somewhat extended description of the internal anatomy of the species and to present our observations on the dorso-ventral axis of the strobila as worked out by us from cross, sagittal and frontal sections of both the mature and gravid proglottides.

THE MATURE PROGLOTTID

(Figs. 1-4)

The mature proglottid, in which all essential anatomical features are well enough defined to be recognizable, has the following dimensions: length from 200 to 250 μ ; width from 1.25 and 1.50 mm; thickness from 700 to 830 μ . The segments, therefore, are strongly ovoid in cross section, being only slightly dorso-ventrally flattened. The margins of the segments are irregular in profile and the strobila is deeply indented at the union of the segments, giving the chain a marked serrated appearance (Figs. 1 and 4). The surface of the segment is covered by a relatively thick cuticle which becomes thinner along the posterior margin of the indented boundary and in the area of the genital pore. Just underneath the cuticle lies a more or less homogeneous matrix of cells from which the cuticle arises. This is followed by a rather



Mesocetoides variabilis Mueller, 1928

FIG. 1. Slightly diagonal, sagittal section (drawn with aid of camera lucida) of mature segment in the region of the genital pore, showing the dorsal position of the genital pore and the ventral position of the ovary.

FIG. 2. Cross section (drawn with aid of camera lucida) of mature segment, showing position and arrangement of muscle layers, position of lateral and median excretory canals on one side and arrangement of the testes about the median excretory canal.

FIG. 3. Diagrammatic sketch, showing the general arrangement of the reproductive system within a single mature proglottid. Ventral view.

FIG. 4. Camera lucida outline of a single mature segment with diagrammatic sketch of both male and female organs, showing their relationship to each other and the positions of the excretory canals. Ventral view.

light band of circular and radial muscle fibers. The radial fibers of this area anastomose with fibers from another band of radial muscles which is arranged with another band of circular muscles in the area of the longitudinal muscles. The conspicuous band of longitudinal fibers, together with the circular and radial bands, make up the principal muscular area of the body (Figs. 1, 2 and 4). This muscular area forms a complete band of muscles surrounding the principal organs of the reproductive and excretory systems. In cross and sagittal sections the band of muscles lies from one-third to one-fourth the diameter of the section from the surface. The longitudinal muscles (L.M) are arranged in definite bundles, each bundle being composed of several strands of fibers and separated from adjacent bundles by the circular and radial fibers. Just mesal to the bundles of the longitudinal fibers lies the most dense concentration of circular (C.M) and radial fibers (Fig. 2, R.M). All important organs except the terminal portions of the genital ducts and the genital pore lie within the area inclosed by the muscular layers. Thus the segment is separated into inner, medullary, and outer, cortical, areas by the bands of muscles.

The male reproductive system. The testes number between 45 and 55 follicles in each side of the segment. They are arranged in irregular rows about the main excretory ducts, the rows completely encircling the main excretory canal throughout the entire length of the canal within the segment. The follicles of the testes (Figs. 2 and 4, T) are round to ovoid in outline and measure up to 60 μ in diameter. They appear to be masses of cell accumulations which are confined within a definite membrane. Although individual sperm ducts were not seen to arise from each testicular body there is evidence in our sections to indicate their presence. A single, rather large sperm duct, the vas efferens (Fig. 3, V.E), arises from each group of the testes and passes postero-mesial from the mid-region of the testicular group to a point just anterior to the ovary. At this point the two vasa efferentia become fused into the formation of a somewhat dilated vas deferens. The vas deferens (Figs. 1 and 3, V.D) becomes convoluted and forms antero-posterior as well as extensive transverse loops in ascending through the medullary portion of the segment to enter the cirrus sac. On entering the cirrus sac the vas deferens becomes more dilated and is thrown into three or four corkscrew-like coils, forming the seminal vesicle (Fig. 3, S.V). Following this the duct develops a thickened, muscular wall, the cirrus, and passes to the opening into the atrium. The muscular portion of the tube is surrounded by a dense mass of deeply staining gland cells. The cirrus is eversible and is seen frequently to protrude through the genital pore for a distance of from 60 to 100 μ . The cirrus pouch (Figs. 1, 3 and 4, C.P) is a thin-walled, weakly muscular structure surrounding the seminal vesicle and cirrus. It measures from 30 to 70 μ in width by 60 to 150 μ in length. The pouch may lie ventro-lateral, ventral, postero- or antero-ventral to the genital pore, however, it is more commonly found lying in an antero-ventral position, especially in the fully gravid proglottid.

The genital pore. The genital pore (Figs. 1, 3 and 4, G.P) is located in the mid-line of the dorsal surface of the segment. It opens to the outside through a pore situated in the floor of a depression in the cuticle in the area of the pore. Internally the pore leads into a flask-shaped atrium. The male duct opens into the atrium from the postero-ventral aspect while the female duct connects with it from the antero-ventral position.

The female reproductive system. The bilaterally lobed ovary (Figs. 1, 3 and 4, O) is situated ventrally, close to the posterior margin of the segment. The two

lobes arch antero-dorso-mesally from a point just ventro-mesally to the area of the testes, in the ventral aspect of the medullary portion of the segment, and are joined mesally by an isthmus of cells. Hence the main body of the ovary lies ventrally to the plane of the longitudinal and transverse excretory canals while the isthmus generally lies dorsal to that plane. The ovary, therefore, due to its arched position, roughly resembles a leaning, inverted U. Each lobe of the ovary is indistinctly separated into two lobes by an indentation near the middle of the lobe (Figs. 3 and 4, O) and is flattened antero-posteriorly (Fig. 1, O). The entire body of the ovary is more or less a loose mass of elongated, ovoid-shaped cells, surrounded by a very delicate and inconspicuous membrane. Each lobe measures from 50 to 60 μ in length, 150 to 180 μ in width and 150 to 230 μ in depth. The distance between the lobes is very variable.

The oviduct arises from the dorsal aspect of that part of the ovarian isthmus which lies dorsal to the plane of the excretory canals. It passes posteriorly from the point of its origin to near the caudal margin of the segment, then turns anteriorly. Almost at once the oviduct develops the conspicuous oöcapt (Figs. 1, 3 and 4, OC). Beyond this the oviduct continues posteriorly to near the caudal boundary of the segment and then swings ventrally and anteriorly and passes anteriorly to about the level of the isthmus before giving rise to the uterus and vagina. From the point of its origin the uterus passes antero-laterally and is soon joined by the common yolk duct (Figs. 3 and 4, C.Y.D). A short distance from this fusion the uterus becomes surrounded by a mass of unicellular gland cells, the shell gland (Fig. 3, S.G). Following this the uterus curves mesally and passes across the segment to the opposite side, passing close in front of the ovary. From the opposite side of the segment the uterus begins an irregular series of transverse loops, ascending through the medullary portion of the segment to a level slightly in advance of the genital pore, where it ends blindly. In making the first transverse swing across the segment the uterus is completely embedded in a dense mass of deeply stained, spindle-shaped cells, the paruterine cells (Figs. 1 and 3, P.C). In segments in this stage of development there are few or no ova to be found within the uterus.

Throughout its entire length the vagina is a slightly convoluted tube (Figs. 1, 3 and 4, V). From its origin to the level of the genital pore the vagina lies ventral to the uterus, but passes around the uterus at the level of the genital pore and arches over the cirrus pouch to enter the atrium from above. As the vagina leaves the ventral position its wall becomes thickened and more muscular and the lumen greatly dilated (Figs. 1, 3 and 4, V). The dilated part of the vagina becomes filled with spermatozoa early in its development and, as Chandler (1942) suggests, probably serves as a receptaculum seminis.

The vitelline glands are composed of two compact lobes, one lobe of which lies on either side of the midline, in the medullary portion of the segment (Figs. 3 and 4, VIT). The lobes occupy a dorsal position within the segment and lie mostly lateral and posterior to the ovary. Each lobe is only about one-half the size of an ovarian lobe. From each lobe of the vitelline glands arises a very delicate yolk duct (Figs. 3 and 4, Y.D) and this passes mesiad, close beside the antero-mesal aspect of the transverse excretory canal, to near the midline of the segment where it joins with its fellow to form a short, common yolk duct. The common yolk duct merges with the uterus a short distance from the origin of the latter.

The excretory system. The excretory system of the mature segment consists of two pairs of longitudinal canals, one pair of which is connected by transverse canals. Both pairs of the canals lie in the medullary portion of the segment and are located on the same medio-frontal plane (Fig. 2). The more lateral pair of the canals consists of two very small, greatly convoluted canals, one on either side of the segment, between the area of the testes and the muscular area (Figs. 2 and 4, L.E). The more median pair, the main excretory canals, consists of two much larger canals. These are only gently undulated throughout their course and lie in the area of the testes, completely surrounded by the testes (Figs. 2 and 4, M.E). The main excretory canals are connected one with the other by the transverse canals. These lie in the area of the junction between adjacent segments (Figs. 1 and 4, T.E.D). Hence a single transverse canal serves the posterior portion of one segment and the anterior portion of the next.

THE GRAVID PROGLOTTID

(Figs. 5-9)

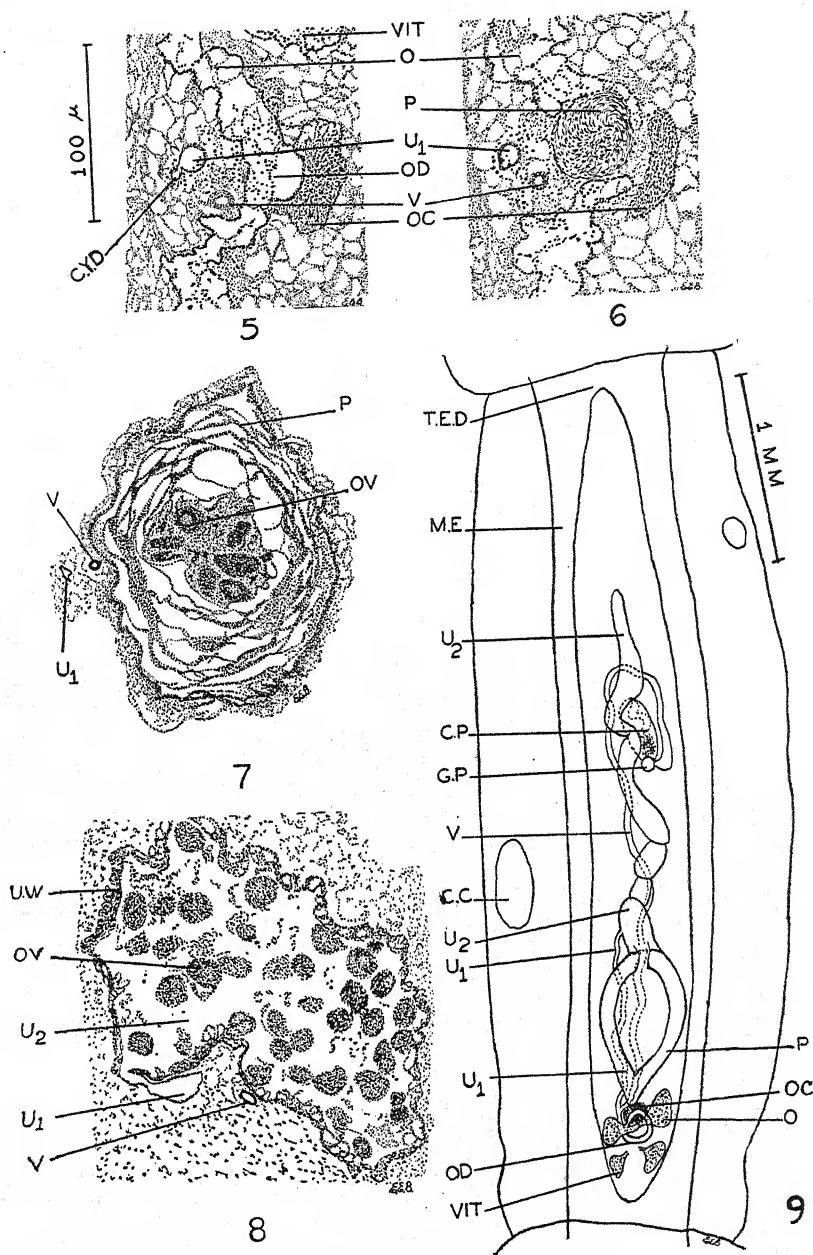
As the segment becomes gravid (gravid in the sense that only the vestige of the female organs and genital ducts can be made out and most of the ova have been crowded into the egg-capsule, paruterine organ) it gradually changes its shape from the condition of the mature segment to, first, a squarish shape and, then, to a greatly elongated one. The segment is decidedly dorso-ventrally flattened. The most gravid segment in the present collection has the following dimensions: length 3.85 mm; width at anterior end 1.13 mm, at genital pore 1.56 mm and at posterior end 1.35 mm.

In the mature segment an occasional calcareous corpuscle is present (in a chain of 34 segments which was sectioned only a single calcareous corpuscle appeared). In proportion to the size of the mature proglottid the calcareous corpuscle is very large, crowding the tissues and organs of the segment to such an extent that the entire segment is distorted. As the segment ripens the calcareous corpuscles become more numerous so that in the fully gravid segment as many as four or five rather large corpuscles may be present. These bodies are not found in every segment and there are usually no more than two to a segment. The calcareous corpuscle generally lies in the cortical portion of the segment, laterally to the excretory canals (Fig. 9, C.C).

The genital pore (Fig. 9, G.P) is dorsal in position and lies in the midline at a level approximating one-third the length of the segment from the anterior end. It measures about 70 μ in diameter.

The cirrus pouch and proximal portion of the vas deferens still persists in the fully gravid segment. The cirrus pouch lies in an antero-ventral position in relation to the genital pore and measures approximately 140 μ in length. Internally the cirrus and seminal vesicle can be made out in sectioned material. The proximal portion of the vas deferens can be traced posteriorly from the cirrus pouch for only a very short distance. In neither the whole mount nor the sectioned material is it possible for us to find any trace of the testes in the fully gravid proglottid.

In the whole mount the uterus and egg-capsule, paruterine organ, are the most conspicuous features within the proglottid. The egg-capsule (Figs. 7 and 9, P) consists of a thick-walled, blind evagination from the uterus, the lumen of which is filled with ova. It measures up to 700 μ in length by 400 μ in greatest width and may lie as close as 600 μ to the posterior margin of the segment. Anterior to the



Mesocetoides variabilis Mueller, 1928

FIG. 5. Cross section (drawn with aid of camera lucida) of gravid segment in region of isthmus of the ovary, showing origin of oviduct, the position of the ovarian lobes, the oöcapit, the vitellaria and the union of the common yolk duct with the uterus. The vitellaria and oöcapit lie dorsal to the median frontal section.

FIG. 6. Same as Fig. 5 except more anterior, at posterior end of paruterine appendage. Part of oöcapit can be seen within the section. Drawn with aid of camera lucida to the same scale as Fig. 5.

FIG. 7. Cross section of paruterine organ in gravid segment. Note vagina (V) on ventral aspect of paruterine organ, between it and the thin-walled, delicate uterus (U₁). Drawn with aid of camera lucida to the same scale as Fig. 5.

egg-capsule the uterus (Figs. 8 and 9, U_2) follows a slightly irregular course along the midline to a level approximately 1.00 mm from the anterior end of the segment, where it ends blindly. The uterus, therefore, terminates at a level in advance of the genital pore. In the gravid segment that part of the uterus anterior to the egg-capsule contains relatively few ova except in the area immediately adjacent to the egg-capsule. Throughout most of its length the gravid uterus becomes greatly kinked and its wall vacuolated.

The more median pair of the excretory canals, the main excretory canals, persists as the excretory system of the gravid segment. This pair of canals (Fig. 9, M.E) becomes greatly expanded, fusing medially at both the anterior and posterior ends of the segment. By their fusion they obliterate the well defined transverse excretory canals of the mature proglottid. The fused longitudinal canals thus form an open vesicle in the anterior and posterior portions of the segment and the cavity of this extends from one segment into the next, passing uninterrupted through the junction between adjacent segments. In neither the whole mount nor the sectioned material are we able to find any trace of the smaller, more lateral pair of excretory canals in the gravid segment.

In sectioned material all of the essential features of the female reproductive system can be distinguished readily. The ovary (Figs. 5, 6 and 9, O) is represented by a bi-lobed, more or less hollow sac. Both of the lateral lobes of the ovary become constricted mesally and the median part arches dorsally and anteriorly from the ventral aspect to about the level of the median plane, forming the isthmus. From the isthmus the oviduct (Fig. 5, OD) arises. It turns posteriorly and ventrally and passes to very near the caudal boundary of the segment before turning anteriorly. The oöcapt (Figs. 5, 6 and 9, OC) is readily seen surrounding the oviduct at the latter's origin from the isthmus. The ascending oviduct (Fig. 9, OD) soon becomes separated into the uterus (U_1) and the vagina (V). Almost at once the uterus receives the common yolk duct (Fig. 5, C.Y.D). From this point the uterus passes anteriorly through an almost straight course, along a somewhat more ventral plane

FIG. 8. Cross section through expanded uterus (U_2) at junction with the thin-walled, delicate part of the uterus (U_1). Expanded uterus lies more lateral while junction of uteri and vagina lie in midline of the segment. Drawn with aid of camera lucida to same scale as Fig. 5.

FIG. 9. Camera lucida sketch of gravid segment, showing diagrammatic details of the female reproductive system. The ovary and vitellariae are drawn as though they were widely separated. Dorsal view.

ABBREVIATIONS

AT—atrium	P.C—paruterine cells
C—cirrus	R.M—radial muscle
C.C—calcareous corpuscle	S.G—shell gland
C.M—circular muscle	S.V—seminal vesicle
C.P—cirrus pouch	T—testis
CU—cuticle	T.E.D—transverse excretory canal
C.Y.D—common yolk duct	U—uterus
G.P—genital pore	U_1 —uterus from oviduct to main part of
L.E—lateral excretory canal	uterus above the paruterine organ
L.M—longitudinal muscle	U_2 —uterus anterior to paruterine organ
M.E—main excretory canal	U.W—uterine wall
O—ovary	V—vagina
OC—oöcapt	V.D—vas deferens
OD—oviduct	V.E—vas efferens
OV—ovum	VIT—vitelline gland
P—paruterine organ	Y.D—yolk duct

than that of the vagina, to a level just anterior to the egg-capsule. Up to this point the uterus is a very delicate, thin-walled tube. Anterior to the egg-capsule the uterus (U_1) suddenly becomes expanded into the egg-storing uterus (U_2). At the point of expansion the uterus gives rise posteriorly to the blind pouch of the egg-capsule and anteriorly to the more opened tube of the egg-storing uterus. In the gravid segment the open part of the uterus contains relatively few ova except in the area adjacent to the egg-capsule. Before the egg-capsule is fully developed the ova are stored temporarily in the expanded uterus (U_2), filling that part of the uterus to capacity. Gradually the ova are crowded from the tubular uterus into the egg-capsule and the expanded uterus undergoes partial atrophy. The wall of the uterus becomes vacuolated, the tube is thrown into a contorted series of kinks, twists and turns, and the lumen is greatly constricted or completely obliterated by the vacuolation of the wall. In the most gravid segment the uterus may become so shrunk that it extends only as far forward as the level of the genital pore.

The egg-capsule is formed as an evagination from that part of the uterus which was embedded in the paruterine cells of the mature segment. Its lumen is derived directly from the lumen of the uterus and the major portion of the wall comes from the paruterine cells. In some segments in our collection the egg-capsule appears to be globular in shape, with a large central cavity, a very short caudal appendage and very thick walls. In other segments from the same chain it appears to be more ovoid in shape, with thinner walls and a longer caudal appendage. Usually the more ovoid the egg-capsule the more pronounced is the caudal appendage. Always the cavity of the capsule is confined to the more globular portion and this is in open communication with the uterus. However as the segment becomes more gravid the opening into the egg-capsule is restricted as the ova are forced into its lumen by the obliteration of the lumen of the expanded uterus. In none of our sections does the cavity of the egg-capsule extend into the caudal appendage although the appendage may be equally as long as the vesicular portion. The appendage may extend as far posteriorly as the level of the ovarian isthmus (Fig. 6, P).

From the point of its origin the vagina passes anteriorly along a plane slightly dorsal to the plane of the uterus (Figs. 5, 6, 7 and 8, V) to the level of the uterine expansion. From this point the vagina follows the expanded uterus anteriorly to a level slightly in advance of the genital pore, remaining throughout this distance closely adherent to the ventral aspect of the uterus. At its most anterior level the vagina turns laterally and dorsally and passes abruptly around the uterus before descending to the genital pore. In passing around the uterus the wall of the vagina becomes thickened and more muscular and the lumen becomes expanded into the cavity which may serve as the seminal receptaculum. The vagina enters the genital atrium from the antero-ventral aspect.

The vitelline glands, like the ovary, are represented in the fully gravid segment by two more or less hollow sacs (Figs. 5 and 9, VIT). These lie in the dorsal aspect of the medullary portion of the segment, partly lateral and posterior to the lobes of the ovary. The vitelline ducts are easily traced in sectioned material.

DISCUSSION

In the present paper we have attempted to give some conception of the details of the anatomy for both the mature and gravid segment of *Mesocestoides variabilis*

Mueller, 1928, from the opossum, *Didelphis virginiana* Kerr. The general features of the anatomy readily adapt themselves to the general plan of the cyclophyllidean cestode but differ from previous conceptions as described for the MESOCESTOIDIDAE in two principal respects: (1) both pair of the excretory canals lie on the same ventro-dorsal plane and (2) the genital pore is dorsal in position.

In standard references on cestode anatomy it is generally stated that the surfaces of the strobila can be designated as dorsal and ventral and that the ovary lies nearest the ventral surface. Usually there are two pairs of excretory canals present and the larger of these is designated as the ventral and the smaller as the dorsal pair.

In our material there are two pairs of longitudinal excretory canals in the mature segment. The larger, more median pair of these is connected by transverse canals and persists throughout the entire strobila. The transverse canals lie in the junction between two adjacent segments and belong to no one segment in particular. In the gravid segment the well defined transverse excretory canals of the mature segment are obliterated by the median expansion of the longitudinal canals. The cavity formed by the expanded longitudinal canals extends from the posterior end of one segment into the anterior end of the next, passing uninterrupted through the junction between the adjoining segments. The smaller, more lateral pair of longitudinal canals do not join by means of transverse canals and even disappear from the segment as the gravid condition is attained. Both pairs of the canals lie on the same dorso-ventral plane in the medullary portion of the segment. In regards to the excretory canals there can be no designation of dorsal and ventral surfaces.

In the material at our disposal the ovary lies nearer to one surface than to the other. Certainly in our material the ovary begins in the area adjacent to the longitudinal muscles of one side and develops the main body of the ovary between that point and the plane of the longitudinal excretory canals, the mid-dorso-ventral plane. Only the ovarian isthmus extends beyond this plane and then only for a sufficient distance for the position to be determined. However the oviduct arises from the isthmus at its most dorsal point. Yet the genital ducts originating from the oviduct swing back to the ovarian side of the segment and only gradually do they resume the more median plane. Therefore, if we can rightfully assume the ovarian side to be the ventral side of the segment, the genital pore is dorsal in position in *M. variabilis* since it opens to the outside through a pore which is situated on the surface opposite that next to which the ovary lies.

SUMMARY

Several incomplete strobilae of a cestode from the opossum, *Didelphis virginiana* Kerr, from Mississippi, have been identified as being identical with *Mesocestoides variabilis* Mueller, 1928. In no instance was a scolex found, although a large number of mature and gravid proglottides is available for study. The material was studied from whole mounts, cross, sagittal and frontal sections.

Attention is drawn to the general topography of the mature and gravid segments, especially to the formation of the egg-capsule and the connections made by the various ducts of the genital systems.

The dorso-ventral axis of the species is discussed from the standpoint of the paired longitudinal excretory canals and the position of the ovary relative to the two surfaces of the segment.

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PHYLLODISTOMUM COATNEYI N. SP., A TREMATODE FROM THE
URINARY BLADDER OF *AMBYSTOMA MACULATUM* (SHAW)

F. G. MESERVE

In September, 1938, nineteen adult salamanders were brought to the laboratory. They were collected in the basement of a summer cottage at Bass Lake, Wisconsin, approximately thirty miles east of St. Paul, Minnesota. Two out of nineteen specimens of *Ambystoma maculatum* were infected.

The following description is based on six out of ten specimens. Measurements were made from four specimens which included the largest and smallest animals. Drawings were made with the aid of the camera lucida.

Phyllodistomum coatneyi n. sp.
(Fig. 1.)

Description: Body thin, flat, unpigmented with posterior part not set off from anterior part. Three times as long as wide with greatest width across the ovarian region approximately half way between the ends. Margins smooth.

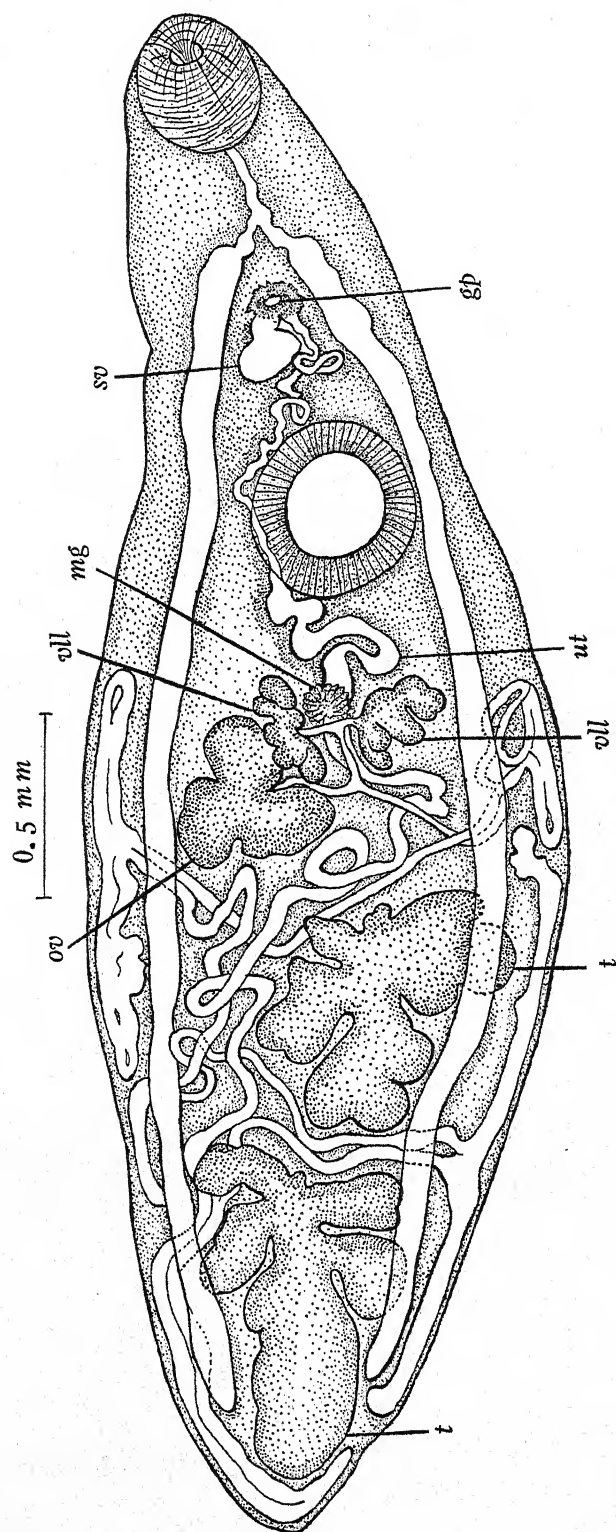
Length 3.3-7.0 mm; greatest width 0.7-1.7 mm. Mouth ventral, sub-terminal. Oral sucker 0.301-0.473 mm in width. Acetabulum at posterior end of first third of body length, 0.455-0.636 mm in width, slightly to right of mid-line. Muscular pharynx lacking. Esophagus relatively slender and almost straight, 0.215-0.344 mm in length. Intestinal crura slightly wavy and relatively large in diameter, left somewhat longer than right, 0.103-0.306 mm from posterior end of body.

Longitudinal axis of sex glands somewhat oblique. Testes irregularly and deeply lobed, in posterior third of body region, oblique. Anterior testis to right of mid-line, 0.490-1.229 mm in length by 0.361-0.860 mm in width. Posterior testis approximately in mid-line, 0.137-0.559 mm from posterior end of body. Seminal vesicle broader than long, 0.094-0.258 mm in length by 0.129-0.412 mm in width, in mid-line to left of anterior end of uterus, opening into a short and wide prostate gland. Vas deferens and excretory vesicle not seen in whole mounts.

Ovary to left of mid-line approximately half way between the two ends, deeply lobed, 0.335-0.559 mm in length by 0.258-0.430 mm in width. Vitelline glands to right and anterior to

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The author wishes to give credit to F. C. Worman, the collector; R. A. Boyce who made the toto mounts; Drs. James E. Lynch and John S. Rankin, Jr. who furnished specimens of related species; Mr. Frank J. Lewis for valuable suggestions; Dr. H. W. Manter who furnished reprints from his private library and Dr. E. W. Price for citations to pertinent literature.

FIG. 1. *Phyllodistomum coatneyi* n. sp.

gp	genital pore	sv	seminal vesicle	ut	uterus
mg	shell gland	t	testis	vll	vitelline gland
ov	ovary				

Drawing made with the aid of a camera lucida.

ovary, deeply lobed and slightly oblique, 0.189–0.464 mm in length by 0.129–0.189 mm in width. Vitelline ducts unite between the vitelline glands to form the common vitelline duct which passes posteriorly to join the oviduct from the left and the uterus from the right. Oviduct leaves the ovary on the right side and passes to right and slightly posterior to join the vitelline duct and uterus. Mehlis' gland between vitelline glands and just anterior to vitelline ducts. Genital pore between oral sucker and acetabulum somewhat posterior to bifurcation of esophagus to form the intestinal crura, in mid-ventral line, 0.232–0.387 mm from the acetabulum and 0.774–0.989 mm from the anterior end. Laurer's canal not seen. Seminal receptacle absent.

Uterus passes posterior to right of seminal vesicle by a tortuous path to left of acetabulum. Just posterior to acetabulum it widens considerably, turns obliquely to the right and after several loops it passes almost straight posterior half way between the vitelline glands. It now turns to the right and after passing posterior to the ovary it turns to the left and passes obliquely posterior to the outside of the left intestinal limb opposite the posterior testis where it forms a loop, then turns to the right across the body of the animal at the level of the posterior third of the body between the testes. It then passes posteriorly between the lateral margin of the animal and the right intestinal limb where it forms a loop and passes forward to the level of the posterior end of the ovary. It now passes to the left between the testes forming another posterior loop on the left outside the intestine. Turning back on itself it passes forward and turns left to lateral edge of animal forming a posterior and anterior loop at level of middle third of body. It then turns posteriorly and passes obliquely posterior to the mid-line and then obliquely anterior to the right forming a V posterior to the ovary. It now forms a short posterior and two short anterior loops opposite the ovary on the right side near the lateral edge of the animal. The uterus now passes obliquely anterior to the left where it joins the oviduct to the right of the ovary. Eggs non-operculate 0.021–0.029 mm in length by 0.015–0.020 mm in width containing embryos.

Host: *Ambystoma maculatum* (Shaw).

Habitat: Urinary bladder.

Locality: Bass Lake, Wisconsin.

Type specimens: Two cotypes will be deposited in the United States National Museum.

P. coatneyi is most like *P. americanum* although there are marked differences. The vitellaria of *P. americanum* are approximately the same size as the ovary whereas in *P. coatneyi* they are only about one half the size of the ovary. The testes of *P. coatneyi* are larger, have more lobes and extend farther forward than in *P. americanum*. In *P. americanum* the uterine loops are more numerous and differ in position, particularly in not extending to the margins of the body; the seminal vesicle is larger and the Mehlis gland is smaller than in *P. coatneyi*. The greatest difference is in the relative shape and size of the eggs which are round and 0.052 by 0.050 mm in *P. americanum* and oval and 0.021–0.029 by 0.015–0.020 mm in *P. coatneyi*.

P. coatneyi is named in honor of Dr. G. R. Coatney of the United States Public Health Service.

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RESEARCH NOTES

A TRAY FOR COLLECTING ANOPHELINE MOSQUITO LARVAE

The larvae of anopheline mosquitoes feed and rest while at the water surface usually in contact with floating or emergent objects. When disturbed, they characteristically swim short distances at the surface or, after swimming downwards a few inches, quickly return to the surface. Because of this behavior, the common practice of collecting larvae by skimming the water in likely breeding spots with a dipper or shallow pan generally gives good results. Frequently, however, larvae of *Anopheles quadrimaculatus*, the important malaria carrier in the southeastern United States, are found among heavy emergent vegetation, brush, or floating debris where surface dipping or skimming is ineffective.

A device now in use in the Georgia Department of Public Health and offering some advantages over the dipper or pan, is a shallow wooden tray, the bottom of which is made of slats. The tray measures about 9 by 16 inches and is about $\frac{1}{2}$ inch deep (see Fig. 1). The slats which

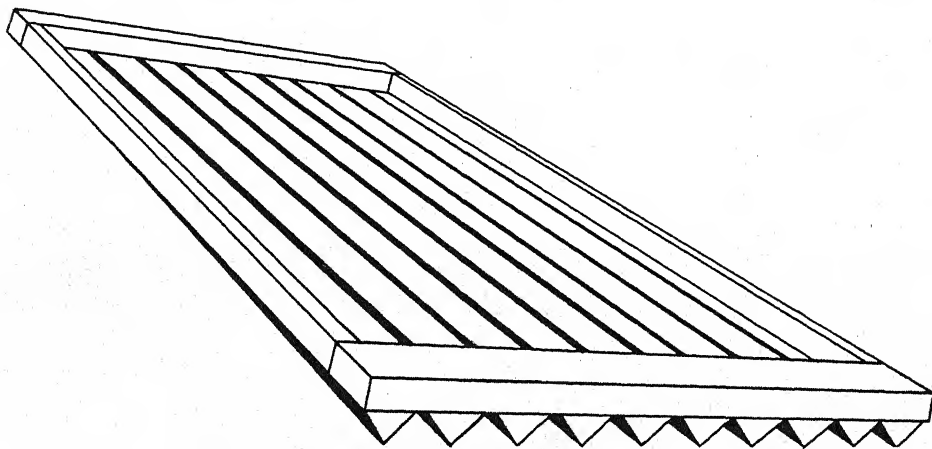


FIG. 1.

are triangular in cross section, are about $\frac{3}{4}$ inch wide and are set $\frac{1}{8}$ to $\frac{3}{16}$ of an inch apart. Thus the bottom of the tray is provided with a flat upper surface and a grill-like under surface. The tray is finished with a heavy smooth coat of white enamel.

In use the tray is placed on floating or emergent objects and pressed down, forcing them below the surface. The larvae quickly work out of the submerged mass and pass with the water through the narrow slits into the tray where they become conspicuous against the white background. Then, while the tray is held down by pressing on it with the specimen bottle, the larvae can be collected with a pipette.

Contribution No. 18, from the Division of Malaria and Hookworm Service, Georgia Department of Public Health, Atlanta, Ga.—PAUL C. BEAVER, *Georgia Department of Public Health, Atlanta, Georgia*.

A PHYSALOPTERAN (NEMATODA) FROM THE DOMESTIC PIG

Numerous specimens of immature *Physaloptera* sp. were recovered from the mucosa of a pig stomach near Manhattan, Kansas, during the month of March. None of the other 30 pigs examined was infected with this worm. These specimens, which at first were mistaken for pieces of straw, were deeply embedded in the mucosa of the stomach and only the posterior end was visible. Of some twenty specimens recovered, the range in length was from 3.4 to 6.5 mm. These worms belong to the genus *Physaloptera* as characterized on the basis of dentition by Schultz (1927, Samml. Helminth. Arbeit, Prof. Skrjabin, Moskva, pp. 287–312). The arrangement of papillae, amphids, and the dentition follows the description of Chitwood and Chitwood (1938, An Introduction to Nematology, Sec. I, Part II, p. 61) for this genus. Generic designation on the basis of uteri groupings (Morgan, 1940, J. Parasitol. 26 Suppl: 40) cannot be determined because of the immaturity of the specimens. Likewise specific diagnosis cannot be

attempted. This appears to be the first report in any country of *Physaloptera* sp. from the pig.—W. M. REID, *Monmouth College, Monmouth, Illinois, and Kansas State College, Manhattan.*

THE TYPE OF *GYROCOELIA* MILLIGANI LINTON, 1927

Identification of certain avian cestodes necessitated better knowledge of *Gyrocoelia milligani*, which was described by Linton (1927, Proc. U. S. Nat. Mus. 70: 1-75) from a sanderling, *Crocethia alba*. The type material, a single slide, was loaned to me through the courtesy of Dr. Benjamin Schwartz.

The type material consists of five fragments from two or more specimens, representing two distinct genera, which have recently (Fuhrmann, 1936, Ann. Parasitol., 14: 261-271) been placed in separate families. The fragments are as follows: (a) A short, broad piece of *Gyrocoelia* female strobila containing nine segments. Evidently one of these segments served for Fig. 190 of Linton's paper (op. cit.). The cirrus pouch (cirrus inverted) (Burt [1939, Spol. Zeylonica 21: 195-208] suspects these structures are actually vaginal pouch and vagina) attains a length of 380 μ . Genital pores alternate irregularly. (b) Another short, broad piece of a *Gyrocoelia* female containing thirteen segments; probably from the same worm as (a), immediately posterior to that fragment. The uterus is plainly circular in several segments, although Linton did not mention this important generic character. Genital pores alternate irregularly. (c) An irregular fragment containing parts of about eleven segments, probably a more anterior piece of the *Gyrocoelia* specimen. (d) A slender fragment containing about twenty segments, but no scolex or evident genitalia. Probably an early piece of the *Gyrocoelia*. (e) Fifteen segments and scolex; a slender, entire specimen of a *Progynotaenia*. The hooks are missing, however, as Linton indicated; the cirri are the only internal organs visible. This specimen, unfortunately, was regarded by Linton as the anterior part of the *Gyrocoelia*, and so figured and described (op. cit.; Figs. 188, 189 and p. 59). But the genital pores are regularly alternate, the suckers are prominent, and the worn, everted cirrus has the characteristic (for *Progynotaenia*) bell-shaped tip, as shown in Linton's Fig. 189. My measurements for this specimen are: Rostellum 66 μ in diameter; sucker 122 μ in diameter; scolex 215 μ in diameter; cirrus pouch 200 to 238 μ long. Definite specific identification seems impossible without rostellar hooks. Fuhrmann (1932, Mem. Univ. Neuchatel 8: 1-381; 173), evidently on the basis of Linton's figure, transferred *milligani* to the genus *Progynotaenia*.

Much of Linton's description was based upon sectioned material, which I have not seen, but which was not part of the designated type. Nevertheless, it may be presumed that this material was *Gyrocoelia*, as Baer pointed out (1940, Parasitology 32: 174-197), because Linton stated that the cirrus passed between the lateral excretory vessels.

Baer (op. cit.) recently referred material from a kilddeer, *Oryechus vociferus*, from Antigua in the West Indies to Linton's species. Because his material and description are excellent, the best taxonomic procedure seems to be as follows: the type of *Gyrocoelia milligani* Linton, 1927, is hereby restricted to those two adjacent fragments described above as (a) and (b) and measuring 4 mm \times 1.2 mm and 6 mm \times 1.3 mm on slide 7892, U. S. N. M. Helm. Coll. The publication of Baer (op. cit.) is taken as the first revision; the specimens referred to therein are assumed identical with the lectotype thus designated. If future extensive examination of the type host, *Crocethia alba*, proves that the *Gyrocoelia* occurring therein is not conspecific with Baer's specimens, redefinition of the species will be necessary.—J. DAN WEBSTER, *Rice Institute, Houston, Texas.*

DISPHARYNX SPIRALIS IN GOLDEN AND RING-NECKED PHEASANTS IN NEW YORK

During October, 1941, we received for autopsy a male and a female golden pheasant (*Chrysolophus pictus*) from a game farm near Mexico, New York (Oswego County). The male bird was found to be infected with several proventricular worms, which were identified as *Dispharynx spiralis*. We believe this is a new host record for this parasite.

In July, 1942, three wild ring-necked pheasant chicks (*Phasianus colchicus*) from eastern Long Island, New York (Suffolk County), were examined. Two of these were parasitized with *D. spiralis*. Recently Madsen (1941, J. Parasitol. 27: 29-34) reported this nematode from ring-necked pheasant chicks in Denmark. He reported that its presence in partridges was a new European host record, but did not claim a new host record for the pheasant, since he cited Cram (1928, U. S. Dept. Agric. Tech. Bull. 49: 1-9) as previously reporting it from pheasants.

It seems possible, however, that Cram's 1928 record represents a lapsus of some sort, since pheasants are not included in her previous list (1927, U. S. Nat. Mus. Bull. 140: 239) or her later paper (1931, U. S. Dept. Agric. Tech. Bull. 227: 1-27). Cuvillier (1937, Rabot. Gel'm.-

Skrijabin: 99), who had access to Cram's records, listed seven galliform hosts for *Dispharynx spiralis* but the pheasant was not among them.—FRANS C. GOBLE AND E. L. CHEATUM, *Research Center, New York State Conservation Department, Delmar, New York.*

A NOTE ON THE LIFE CYCLE OF *AUSTRALORBIS GLABRATUS* (SAY, 1818)
PILSBRY, 1934, A SNAIL INTERMEDIATE HOST OF
SCHISTOSOMA MANSONI

Shortly after hatching from eggs, fourteen specimens of the snail, *Australorbis glabratus* (syn. *Planorbis guadeloupensis* Sowerby, 1822), were isolated, one to a battery jar which contained three liters of water and some aquatic plants. The water of these cultures had a pH between 7.5 and 8.5. It was found that water samples collected in places where there were large populations of these snails in the field also had a low alkalinity. The aquatic plant used in the cultures was either *Naias guadeloupensis* or *Eichornia azurea* which species are commonly found in the natural habitat of *A. glabratus* in Venezuela. Small pieces of fresh lettuce were added to the cultures twice a week and the water was renewed as frequently as was necessary. The battery jars were covered with glass plates in order to reduce evaporation. The cultures were maintained in a place supplied with ample indirect sunlight and here the temperatures of the water of the cultures ranged from 20° to 29° C. Since this snail is disc-shaped and grows in a concentric fashion, the diameter was used as an index of its growth. Table 1 includes some observations which were made on the fourteen snails during their first year of life in the laboratory.

The data in the table emphasize the variations in the development and egg-production of these fourteen snails which came originally from only three different groups of eggs. Oviposition, in the snails began from 79 to 190 days following hatching. The diameter of the snails at that time ranged from 6 to 13 millimeters. The total number of eggs deposited by each snail during the first 12 months of life varied from 55 to 1045. At the end of the first year the snails were still growing and laying eggs in the cultures. The presence of one or the other species of plants mentioned did not seem to effect the growth or egg-production of the snails. There did not seem to be any correlation between the number of eggs deposited by each snail and the age of the snail at its first oviposition.

As described by Hoffman and Faust (1934, Puerto Rico J. Pub. Health and Trop. Med. 9: 228-282), it was found that *A. glabratus* laid groups of from 1 to 30 eggs embedded in a translucent albuminoid substance and each egg had a length of about 1 millimeter. With the temperatures ranging from 20° to 29° C in the laboratory, it took from 8 to 12 days for the eggs to develop and hatch. In three different tests it was found that 64% of the eggs hatched.

TABLE 1.—The development and egg-production of the snail, *Australorbis glabratus*, during its first year of life

Snail	First oviposition		Total number of eggs produced	Diameter at end of first year (mm)
	Age (days)	Diameter (mm)		
1	79	8.0	1045	15.0
2	81	7.0	885	14.5
3	101	7.3	158	15.5
4	139	8.0	374	16.0
5	139	9.0	960	15.0
6	145	9.5	644	16.0
7	149	6.0	367	13.5
8	156	8.0	399	17.0
9	156	10.0	451	16.0
10	172	13.0	57	18.0
11	172	9.0	828	15.5
12	175	10.5	162	15.5
13	178	14.0	182	18.0
14	190	13.0	55	19.0

The observations made in the laboratory and in the field, indicate that the life cycle of this snail continues throughout the year in Venezuela. By comparing the average diameter of the *A. glabratus* in the year-old cultures in the laboratory with the diameter of the largest specimens of this species which were found in the field, the duration of the life of *A. glabratus* may be estimated as one year and a half to two years.—GEORGE W. LUTTERMOSER, *Instituto Nacional de Higiene, Caracas, Venezuela.*



ALBERT HASSALL (1862-1942)

IN MEMORIAM

ALBERT HASSALL (1862-1942)

On September 18, 1942, Dr. Albert Hassall, parasitologist and bibliographer, passed away at Bangor, Me., as the result of a heart attack following a surgical operation, and was buried September 23, at Collington, Md. He is survived by one sister, two sons and three daughters.

Albert Hassall, the only son of Major Thomas and Elizabeth Hassall, was born February 12, 1862, in Woolwich, Kent, England. After receiving his early education in private schools, he entered the Royal Veterinary College in London and was graduated as a Member of the Royal College of Veterinary Surgeons from that institution in 1886. While in London he had as one of his professors the late Dr. T. Spencer Cobbold, at that time England's most distinguished parasitologist, whose influence over this young veterinary student was largely responsible for his future career. After his graduation, Hassall's father wished him to enter the Colonial Service; this did not appeal to Albert, so he came to the United States in 1887 and almost immediately entered the infant Bureau of Animal Industry. He was appointed veterinary inspector and assigned to duty to assist in the eradication of contagious pleuropneumonia, a destructive disease of cattle that had gained a foothold in this country a few years previously. On April 30, 1890, he entered veterinary practice in Baltimore, but Cobbold's influence had been too profound and on March 7, 1891, he reentered the government service as assistant to the late Dr. Cooper Curtice, who was the Bureau's first parasitologist. Following the resignation on May 31, 1891, of Dr. Curtice and the appointment on June 3 of the same year of Dr. Ch. Wardell Stiles, in charge of the Zoological Laboratory, Hassall was Stiles' principal assistant until November 29, 1902, when he was transferred to Chicago, Ill., for duty in the Meat Inspection Service. Upon the resignation of Stiles to become Chief of the Division of Zoology of the Hygienic Laboratory of the United States Public Health and Marine Hospital Service, now the National Institute of Health, and of the appointment of the late Dr. B. H. Ransom to succeed him in the Bureau, Hassall returned to Washington and on April 14, 1904, was assigned to duty in the Zoological Division. At his own request he was assigned to bibliographic work, continuing the Index-Catalogue which he had started several years previously. Hassall served from 1905 to 1910 as assistant in zoology and from 1910 to 1924 as assistant zoologist under Ransom, and from 1928 to 1932 as senior zoologist and Assistant Chief of the Zoological Division under the late Dr. M. C. Hall. In 1932, having reached retirement age, Hassall was given a two-year extension to continue his work, but owing to the Economy Act he was retired on June 30 of that year. For several months Hassall continued to work without compensation and on July 16, 1934, he was given the title of Collaborator in order that he might have official status in the Bureau and to permit the publication under his name of the Index-Catalogue of Medical and Veterinary Zoology which was being reissued.

Hassall's scientific interests date from his student days under Cobbold. In his earlier years he was an indefatigable collector and his extensive collection is deposited in the U. S. National Museum, forming a substantial part of the nucleus of the Helminthological Collections of that institution.

The Index-Catalogue of Medical and Veterinary Zoology, with which he was most closely identified, was started by him shortly after he became Stiles' assistant. One of his duties was to look up references to the literature for his chief, and since these references were, at that time, difficult to secure, he conceived the idea of saving the reference cards so that they might be readily available for future use. With the passage of time the accumulation of references was expanded to meet the needs of the increased staff of the laboratory and then to meet the requirements of a growing and expanding science of parasitology.

Hassall published only about 15 papers under his own name, the greater part of his work appearing under the joint authorship of Stiles and Hassall. This was partly due to his lack of desire for personal glory and partly because of a custom prevailing at the time he entered the government service which decreed that heads of departments in educational institutions and in government divisions and offices were, ipso facto, senior authors of all papers from their respective units. Hassall, however, persistently refused to occupy any rank lower than that of junior author or to bask in reflected glory of big name authors, maintaining that he would "never play third fiddle in any orchestra." As a result of this attitude, a few monographic papers which embodied a large amount of his work do not bear his name. The joint authorship of Stiles and Hassall ended with the publication in 1929 of Bulletin 152 of the Hygienic Laboratory, U. S. Public Health Service, entitled "Key-Catalogue of Parasites Reported for Primates (Monkeys and Lemurs) with Their Possible Public Health Importance." At this time the Damon and Pythias of parasitology agreed to cease joint publication, with the understanding that the Key-Catalogue series was to be published by Stiles and the Index-Catalogue series by Hassall.

For the most part Hassall's published work was that of compilation, a type of endeavor that does not gain the plaudits of the thoughtless, or of the neophyte having his initial fling in the scientific world, or of those who have failed to acquire wisdom with age, even though it saves these individuals incalculable hours of labor. Furthermore, Hassall's characteristic thoroughness assures the users of his compilations that, so far as humanly possible, the information contained in them is complete and reliable.

Some idea of the thoroughness of his work may be gained from the following verses written by the late Dr. N. A. Cobb and presented at a birthday dinner given in honor of Dr. Hassall in 1932:

The Recording Angel (Dr. Albert Hassall)

When I meet the recording angel
Of the mighty B. A. I.
My sins rise up before me
And smite me hip and thigh.
For I know there's no escaping
His well known, eagle eye.
He spots my every error
And intends to till I die.

And he writes them in his Files,
Inscribes them in his Files.

If I have two MS species
Of the same generic form,
And because I'm absent-minded
Or my brain is in a storm,
I name them in terms identic
For my old friend Dr. Dorm,

It's, "What the hell you doing?"—
Or something just as "warm."

So this escapes his Files,
Thanks be, escapes his Files.

But if I rediscover
A form from the misty past
And mistakenly rename it,
I never hear the last
Of how I've bungled the works up
With the "wrenches" I have cast,
"At your abysmal ignorance
The whole world stands aghast."

For he'll have it in his Files,
He'll have it in his Files.

If I named a species "*minor*"
In the long, long, long ago,
And after time's gone flying
What seems an age or so,—
I apply it to a brother species,
I quaff the bitterest woe!
It's "Cobb, your case is hopeless,
I'd make my will and go!"

He'll have it in his Files,
He'll have it in his Files.

And when I land in Tophet
I shall hear it relayed down
From the land I left behind me,
As I sizzle and I brown;—
"There's one thing I'd forgotten,
That genus from beyond the town,—
The name you went and gave it
Is an *adjective*, not a noun."

It's up there in his Files,
It's up there in his Files.

As a result of his bibliographic work, Hassall became an authority on the subject of zoological nomenclature. He had a retentive memory and his accumulated knowledge and experience were always available to his associates and to all who genuinely sought information. He was a thoroughgoing rugged individualist who wore no man's collar and scraped no man's boots; he was a stickler for truth and admitted no compromise for the sake of expediency. In spite of his ruggedness, he was a kindly gentleman and a sincere friend whose greatest pleasure was in helping others.

Hassall took little interest in scientific societies, owing largely to the fact that he was a farmer during his free hours. However, he was an early member of the Helminthological Society of Washington, served as its president in 1921-22, and was elected to life membership in 1931.

For his distinguished service and in recognition of his contributions to science and veterinary medicine, the Royal College of Veterinary Surgeons in London awarded to Dr. Hassall the Steel Memorial Medal in 1922, given for pre-eminent work.

May he rest in peace in the congenial company of those whose names he immortalized in the Index-Catalogue of Medical and Veterinary Zoology.—E. W. PRICE
AND G. DIKMANS, *Zoological Division, U. S. Bureau of Animal Industry.*

AMERICAN SOCIETY OF PARASITOLOGISTS

THIRTY-FIRST COUNCIL MEETING, NEW YORK CITY

JANUARY 9, 1943

The meeting of the Council of the American Society of Parasitologists was called to order by Dr. Henry E. Meleney, President of the Society, at 2:00 PM, January 9, 1943, in the offices of the Department of Preventive Medicine, New York University, 341 East Twenty-fifth Street, New York City. The following members of the Council were present: D. L. Augustine, R. M. Cable, J. T. Culbertson, R. W. Glaser, H. E. Meleney, G. F. Otto, L. E. Rozeboom, N. R. Stoll, and H. W. Stunkard.

I. REPORTS OF OFFICERS

1. *Secretary (J. T. Culbertson)*: As of December 31, 1942, there were 534 persons on the membership roll of the Society, of which 463 lived within and 71 lived outside continental United States. Of the total number on the roll, 437 were members in good standing and 99 were delinquent for from one to three years. Of those in good standing, 396 lived within and 41 outside continental United States. Fifty new members were elected during the year, of which number 45 lived within and 5 without the United States. Four members died during the year: Dr. Robert Hegner of Baltimore, Maryland, Dr. William James of Ancon, Republic of Panama, Dr. Marcus W. Lyon, Jr., of South Bend, Indiana, and Dr. Winfield C. Sweet of La Paz, Bolivia.

Upon motion the Secretary's report was accepted and placed on file.

2. *Treasurer (L. E. Rozeboom)*: For the fiscal year 1942 (Dec. 2, 1941 to Dec. 7, 1942):

Receipts

Balance on hand, Dec. 2, 1942		\$1805.83
Collected during current year		
Member dues applying 1942	\$ 975.31	
" " " 1943	933.95	
Advance dues	31.50	
		\$1940.76
Subscriptions applying 1942	1734.56	
" " " 1943	360.00	
Advance subscriptions	4.50	
		2099.06
Back nos. and vols. sold	373.55	
25-volume index sales	180.60	
Contributions from members	224.00	
Advertisements	65.50	
Author charges	123.77	
Miscellaneous	49.52	
		5056.76
Loan from Princeton Fund		400.00
		\$7262.59

Expenditures

Printing 25-vol. index, balance	\$ 754.78	
" Journal, 1941 account	754.14	
" " 1942 "	2532.07	
		4040.99
Expenses in office of Chm. Ed. Comm.	111.43	
" " " Secretary	162.44	
" " " Treasurer	395.34	
Miscellaneous	27.24	
		- 4737.44
Balance on hand Dec. 7, 1942		\$2525.15

Upon motion the Treasurer's report was accepted, subject to audit, and placed on file.

Council voted that the Treasurer's report be published in summary form, covering all business transacted during the fiscal year 1942, but omitting all allocations. Council also voted that the Treasurer be instructed to account in reports for future years for all moneys on hand, for all business transacted, and for the cash balance at the end of the then current fiscal year, giving due regard for essential itemization.

II. REPORTS OF COMMITTEES

1. *Custodian of the Secretarial Fund (N. R. Stoll)*: For the fiscal year Dec. 1, 1941 to Dec. 1, 1942:

Total liquid assets	\$ 475.79
Book value, subsidiary assets	816.67
	<hr/>
Total	1292.46
Total, preceding year	1276.77
	<hr/>
Increase, total assets current year	15.69

Upon motion, the report was accepted, subject to audit, and placed on file.

2. *Chairman of the Editorial Committee (N. R. Stoll)*: During 1942, the six regular numbers of Volume 28 of the JOURNAL OF PARASITOLOGY containing 506 pages and the December Supplement containing 50 pages were issued on schedule. The regular numbers contained 85 articles and research notes and 8 miscellaneous reports. The interval between "received for publication" and date published averaged 11.0 months in 1942 for regular articles (10.7 in 1941), and 10.3 months for research notes (9.1 in 1941). It was noted that manuscripts submitted in the years 1938 to 1942 totalled for the successive years, respectively, 84, 95, 107, 100, and 79.

Three complications in publishing the journal were mentioned: (1) the loss of members and subscribers in part through the government prohibition of export to certain countries; (2) the requirement that each issue in proof form be cleared through the Office of Censorship; and (3) possible shortages of materials.

Certain changes in format begin with the February, 1943, issue for a two-year experimental period: (1) the type page will be slightly enlarged; and (2) blank or partly blank pages will when possible be omitted. Beginning with the next volume, authors will be charged for pages in excess of 10, instead of 20 as at present.

Upon motion, the report was accepted and placed on file.

3. *Auditing Committee (D. L. Augustine and R. M. Cable)*: The Auditing Committee approved the reports of the Treasurer and of the Custodian of the Secretarial Fund.

Upon motion the report of the Auditing Committee was accepted and placed on file.

III. REPORTS OF REPRESENTATIVES OF THE SOCIETY

1. *Representative to the Union of American Biological Societies (G. L. Graham)*: Prof. E. G. Butler is the new president of the Union of American Biological Societies. Plans are under way to expand the activities of the Union, particularly with regard to advising with respect to the increasing number of applicants for Latin-American fellowships to this country. It is recommended that "the American Society of Parasitologists adopt the policy and procedure of voting a regular annual contribution to the Union of sufficient amount to be of real assistance in conducting their affairs and that said contribution be made without request on the part of the officers of the Union." By vote of Council the report of the representative was accepted, with the reservation that no financial contribution be made at this time for the support of the Union or of Biological Abstracts.

IV. NEW BUSINESS

1. *New Members*: Council voted membership in the Society upon four applicants: Lester L. Bissinger, Department of Zoology, University of Minnesota, Minneapolis, Minnesota; Margarita B. Hollis, Instituto de Biología, Chapultepec, Mexico, D.F.; C. Clayton Hoff, Department of Zoology, Quincy College, Quincy, Illinois; and Morton C. Kahn, Department of Preventive Medicine, Cornell University Medical College, New York City.

2. *Next Annual Meeting*: The selection of a meeting place for the Society in 1943 was deferred by vote of Council until more information with respect to such a meeting be available.

It was suggested by G. F. Otto that if war conditions should prevent the meeting of the entire Council at the end of 1943, the Society's business be conducted by mail, possibly following a meeting of those Council members living in the New York area.

3. *Program Committee*: Council voted to establish an Advisory Committee on Program and Abstracts consisting of the Secretary (Chairman), the Chairman of the Editorial Committee, and the President of the Society.

4. *New Business Related to JOURNAL OF PARASITOLOGY*:

(1) *Sales of the Journal to American Library Association's "Committee on Libraries in War Areas"*: Council voted to approve future sales of the JOURNAL at four dollars per volume to the Committee on Libraries in War Areas.

(2) *Reprints of Abstracts in December Supplement*: Council voted that recommendation be made to the Editorial Committee of the JOURNAL that no reprints of individual abstracts printed in the Supplement be made available.

(3) *Insurance of Journals in Storage*: Council voted to authorize the Treasurer to insure journals now stored at the Johns Hopkins University School of Hygiene and Public Health against damage by fire, water, or other hazards including those related to the war. Details of the procurement of this insurance were left to the discretion of the Treasurer.

(4) *Custodian of the Journal Stock*: Council voted to authorize the Treasurer to appoint a Custodian of the Journal stock, should the necessity arise.

(5) *Miscellaneous*: Council agreed with the suggestion of the Chairman of the Editorial Committee that the basic issue of the JOURNAL be raised from 1000 to 1100 copies.

5. *Election of Officers, etc.*: Following extended discussion, Council voted (seven votes for and one against) that present officers and members of Council be continued in office until the next meeting of the Society. Accordingly, officers for 1943, or until the Society's next meeting, are: *President*, H. E. Meleney, *Vice-president*, R. W. Glaser, *Secretary*, J. T. Culbertson, and *Treasurer*, L. E. Rozeboom. Likewise, E. W. Price and E. R. Becker, who were originally scheduled for retirement from Council, will continue as Council members.

The Nominating Committee (W. W. Cort, Chairman) for the Selection of a New Editorial Committee for the JOURNAL recommended the following: H. W. Stunkard (Chairman), W. A. Riley, and D. H. Wenrich. The new Editorial Committee would serve for five years assuming responsibility with Volume 30. Council voted to accept the recommendations of the Nominating Committee.

For the three annual vacancies on the Editorial Board of the JOURNAL, Council voted to elect R. W. Glaser, B. G. Chitwood, and P. P. Levine as successors to C. B. Philip, A. C. Chandler, and E. E. Tyzzer, respectively, whose terms had expired.

President H. E. Meleney appointed E. W. Price Chairman of the Nomenclature Committee to succeed P. D. Harwood, who had resigned. The President reappointed the present representatives of the Society on the Council of the American Association for the Advancement of Science, and to the Union of American Biological Societies.

The meeting was adjourned at 9:45 PM. From 5:45 until 7:30 PM the meeting had been recessed in order that the members of Council entertain the President of the Society at dinner in the Gramercy Park Hotel, New York City.

Respectfully submitted,

JAMES T. CULBERTSON, *Secretary*.

The Journal of Parasitology

Volume 29

AUGUST, 1943

Number 4

DIPETALONEMA ARBUTA N. SP. (NEMATODA) FROM THE PORCUPINE, *ERETHIZON DORSATUM* (L.)

PAUL R. HIGHBY¹

The filarioid nematode commonly found in the peritoneal cavity of *Erethizon dorsatum* (L.) has been identified by Canavan (1929 and 1931) and Jellison (1933) as *Dipetalonema diacantha* (Molin, 1858). It was first identified as *Filaria diacantha* Molin, 1858 by Boulenger (1920) who referred it to the genus *Acanthocheilonema* Cobbold, 1870 as emended by Railliet, Henry, and Langeron (1912). This genus was considered to be a synonym of *Dipetalonema* Diesing, 1861 by Yorke and Maplestone (1926).

F. diacantha recently has been redescribed and made the type of a new genus, *Molinema* Teixeira de Freitas and Lent, 1939. Specimens from *E. dorsatum* available to me do not agree morphologically with *M. diacantha* as redescribed from *Coendou villosus* (Cuv.) by these authors. Since specimens from this host or from the type host of Molin's species, *Coendou prehensilis*, are not available to me I must accept the Brazilian workers' redescription of the species objectively and must conclude that the specimens from *E. dorsatum* represent a previously unrecognized species of *Dipetalonema*; for it the name *D. arbuta* is proposed.

Dipetalonema arbuta n. sp.

(Figs. 1-7)

Description: Dipetalonema: Oral opening round; 8 cephalic papillae; amphids lateral. Ventrolateral terminal caudal conical processes prominent in both sexes.

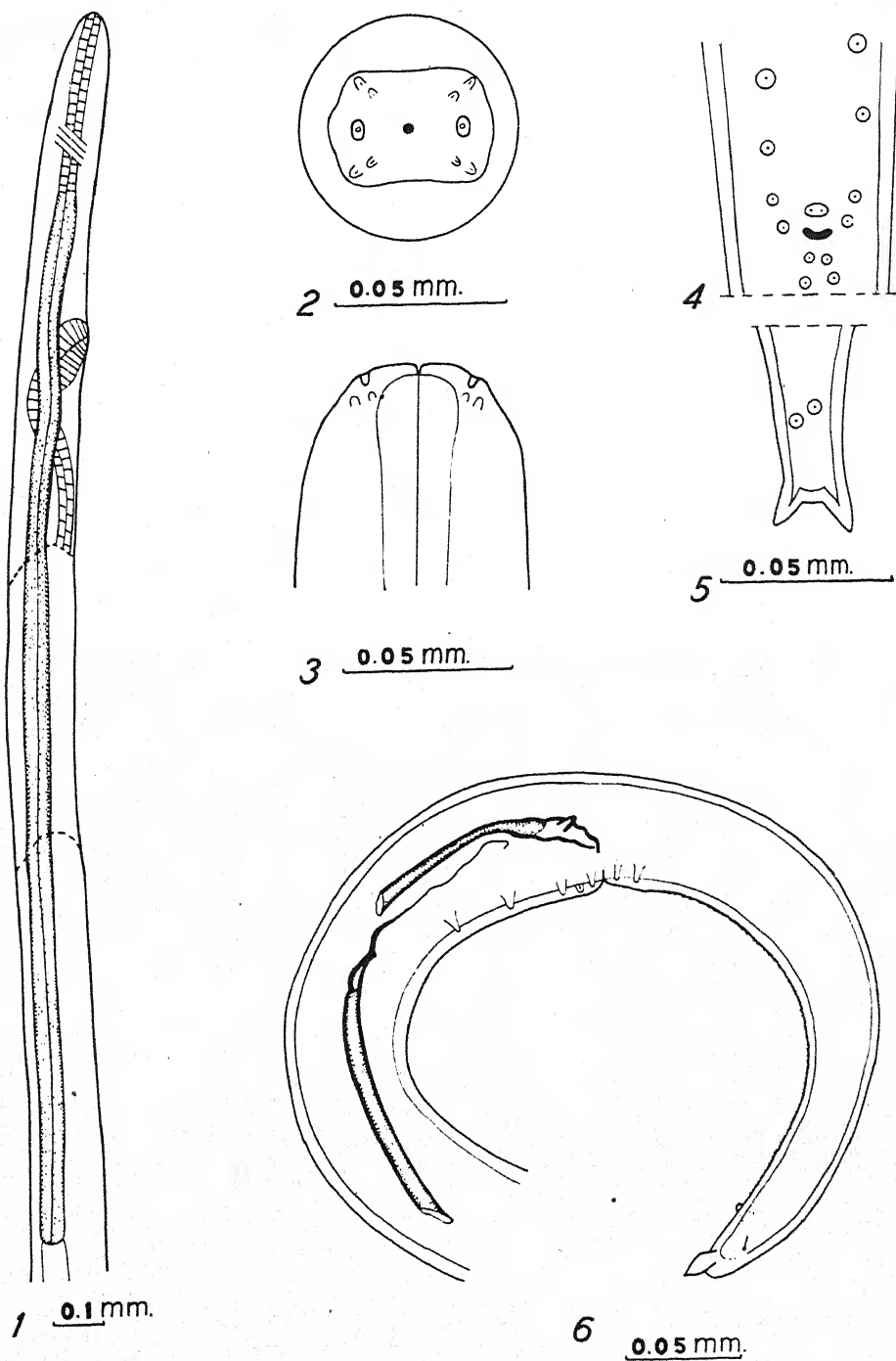
Male 22 to 37 mm (31 mm)² long by 0.17 to 0.21 mm wide. Esophagus 2.20 to 2.72 mm (2.39 mm) long; anterior portion 0.39 to 0.49 mm (0.43 mm) long by 0.025 to 0.037 mm (0.032 mm) maximum width; posterior portion 1.83 to 2.23 mm (1.98 mm) long by 0.043 to 0.070 mm (0.055 mm) maximum width. Nerve ring 0.20 to 0.24 mm from anterior extremity. Cloacal aperture 0.23 to 0.33 mm (0.27 mm) from caudal extremity. Pendunculated genital papillae 7 pairs, 4 preanal and 3 postanal, and additional medioventral double one between the 3rd and 4th preanals, the last preanals in adanal position, the last postanals 0.016 to 0.025 mm from caudal extremity. Long spicule 0.212 to 0.225 mm (0.220 mm), anterior portion tubular, posterior filiform, hooked. Short spicule 0.112 to 0.125 mm (0.119 mm), stout, tubular, bent in middle, enlarged posterior portion, hooked. Ratio of spicules 1:1.8 to 1:2. Gubernaculum absent. Phasmids present. Posterior portion of body spiralled in 4 to 6 loops. Longitudinally striated transverse ridges on ventral side in coiled region of body anterior to cloacal aperture, 0.023 to 0.029 mm apart anteriorly, 0.008 to 0.012 mm apart posteriorly.

Female 58 to 82 mm (68 mm) long by 0.25 to 0.33 mm (0.27 mm) maximum width. Esophagus 2.07 to 2.77 mm (2.35 mm) long; anterior portion 0.28 to 0.48 mm (0.34 mm) long by 0.035 to 0.047 mm (0.042 mm) maximum width; posterior portion 1.77 to 2.29 mm (2.01 mm) long by 0.064 to 0.082 mm (0.072 mm) maximum width. Nerve ring 0.19 to 0.26 mm (0.23 mm) from

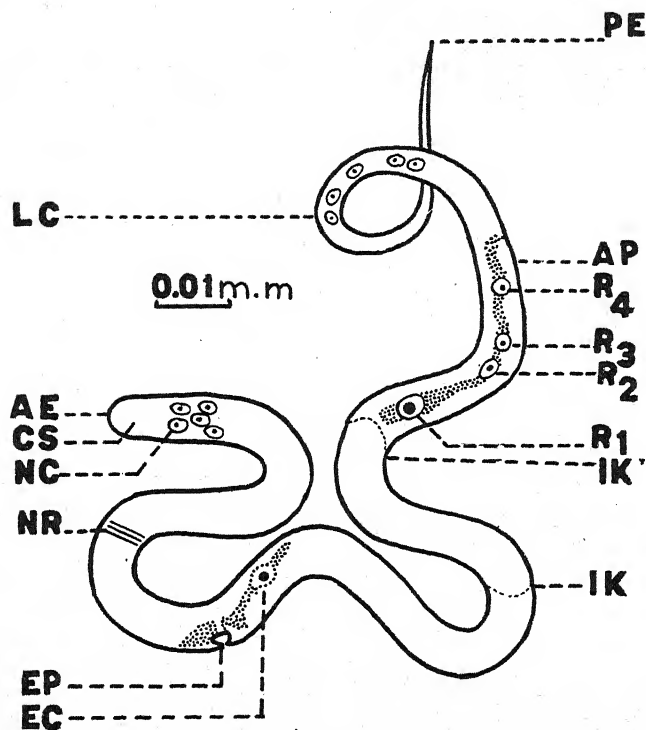
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¹ From the Department of Zoology, University of Minnesota, Dr. William A. Riley, adviser.

² The figures in () represent average values.



All figures were drawn with the aid of a camera lucida.
 FIGS. 1-3. Adult female: 1, anterior end, lateral view; 2, anterior extremity, en face view; 3, anterior extremity, dorsal view.
 FIGS. 4-6. Adult male: 4, genital papillae of cloacal region, ventral view; 5, tip of tail, ventral view; 6, caudal region, lateral view.



Dipetalonema arbuta n. sp.

FIG. 7. Microfilaria of *Dipetalonema arbuta* n. sp., drawn with aid of camera lucida.

- | | |
|-----------------------------------|---------------------------------------------------------------------|
| AE—anterior extremity | IK'—posterior end of "Innen Körper" |
| AP—anal pore | LC—last cell of nuclear column |
| CS—cephalic space | NC—first cells of nuclear column |
| EC—excretory cell | NR—nerve ring |
| EP—excretory pore | PE—posterior extremity |
| IK—anterior end of "Innen Körper" | R ₁ –R ₄ —1st, 2nd, 3rd, and 4th rectal cells |

anterior extremity. Anus 0.20 to 0.25 mm from caudal extremity. Phasmids present. Vulva 0.45 to 0.85 mm (0.70 mm) from anterior extremity.

Microfilariae not sheathed, 0.280 to 0.297 mm (0.288 mm) long by 0.006 mm wide (20 specimens measured in aqueous Azur II). Head obtuse. Body slender. Tail long, slender, and pointed. Cephalic space longer than its width. "Innen Körper" begins 132 to 155 μ from the anterior extremity and continues to the anterior border of the first rectal cell. Second and third rectal cells midway between the first and fourth. Fourth rectal cell just anterior to anal pore. Last nucleus of the nuclear column about midway between anal pore and posterior extremity. Last 4 to 6 nuclei typically arranged in tandem, attenuated, and scattered with spaces between. Position of anatomical points in terms of distance from the anterior extremity expressed in percentages of the worm's total length (av. of 20 specimens): length of cephalic space 2.3 per cent, width of cephalic space 1.7 per cent, nerve ring 20.4 per cent, excretory pore 27.1 per cent, excretory cell 31.9 per cent, first rectal cell 65.0 per cent, anal pore 74.5 per cent.

Host: *Erethizon dorsatum* (L.).

Location: Adults in pleural, peritoneal and pericardial cavities. Microfilariae in blood and body fluids.

Locality: Northern Minnesota, U. S. A.

Type specimens: Holotype male, U. S. N. M. Helm. Coll. No. 45037; paratypes, 3 males, 3 females, and dissections, No. 45038. Other paratypes in the Department of Zoology, University of Minnesota.

Remarks: 6 male and 6 female adults were measured for the above descriptions except for body length for which 10 males and 20 females were measured.

D. arbuta may be distinguished from *M. diacantha* by certain differences. *M. diacantha* has 2 lips with 6 papillae, *D. arbuta* has no lips but has 8 cephalic papillae. The males of *M. diacantha* are from $1\frac{1}{3}$ to 2 times as long as *D. arbuta* males, but the females of the former are only slightly larger. In *M. diacantha* the vulva is 0.83 to 1.33 mm from the anterior end, in *D. arbuta*, 0.45 to 0.85 mm. The ratio of the spicule lengths in *M. diacantha* is 1:1.3 to 1:1.4, in *D. arbuta*, 1:1.8 to 1:2. *M. diacantha* has 4 pair of preanal and 1 pair of postanal papillae, *D. arbuta* has 4 preanal pair, 1 median double preanal, and 3 postanal pair.

The microfilariae of *M. diacantha* are 0.20 to 0.23 mm long and 8μ wide, sheathed and with obtuse extremities; those of *D. arbuta*, 0.280 to 0.297 mm long and 6μ wide, *unsheathed* and with a long pointed tail.

The development in various species of mosquitoes has been traced and will be reported later.

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MOSQUITO VECTORS AND LARVAL DEVELOPMENT OF
DIPETALONEMA ARBUTA HIGHBY (NEMATODA)
FROM THE PORCUPINE, *ERETHIZON*
DORSATUM

PAUL R. HIGHBY

The porcupine, *Erethizon dorsatum* (L.) in Minnesota is commonly parasitized with *Dipetalonema arbuta* Highby, 1943 in its peritoneal cavity and *Dirofilaria spinosa* Canavan, 1929 in its dorsal subcutaneous connective tissue. These worms were previously reported from this host by Boulenger (1920), Canavan (1929 and 1931) and Jellison (1933). I examined 28 porcupines from northern Minnesota and found 10 infected with both species, 2 with *D. spinosa* only, 10 with *Dipetalonema arbuta* only, and 6 with no filarioids.

Microfilariae of these filarioids may be found in the infected porcupine's blood, both peripheral and central, the pericardial, pleural and peritoneal fluids, the urine, the lungs and liver. The microfilariae of *D. arbuta* were recovered also from the mixed amniotic fluid and blood of a fetus removed from an infected porcupine.

Microfilaria arbuta may be distinguished from *Mf. spinosa* in both wet and dry preparations by three characters:—*Mf. arbuta* has a cephalic space which is longer than its width while that of *Mf. spinosa* is shorter than its width; the terminal nuclei at the posterior end of the nuclear column of *Mf. arbuta* present a more scattered appearance than the more compact arrangement seen in *Mf. spinosa*; and in *Mf. arbuta* an "Innen Körper" is present, but none is apparent in *Mf. spinosa*. *Mf. arbuta* was described in detail by Highby (1943).

EXPERIMENTS TO DETERMINE SUSCEPTIBLE VECTORS

Feeding experiments were attempted with laboratory-reared native mosquitoes and simuliids without success for the insects failed to feed. However, an exotic species, *Aedes aegypti*, reared from eggs imported from Florida fed avidly on the porcupine, and in this species development of *Dipetalonema arbuta* was completed.

Wild adult mosquitoes were used in further feeding experiments. Of 337 mosquitoes dissected after feeding upon porcupines infected with *D. arbuta* 52 per cent became infected. That these infections were contracted from the experimental source of infection may be inferred by comparison with the natural incidence of filariasis in wild mosquitoes from this locality.

A series of dissections of captured local mosquitoes experimentally fed was made in connection with this and subsequent experiments. These experimental mosquitoes represented 12 species of demonstrated susceptibility to filarioid infections. Nine of the 12 species had been shown to be susceptible to the development of *Dirofilaria immitis* by Yen (1938). Specimens of three other species were also used experimentally of which two were shown to be susceptible and one probably susceptible to *D. arbuta* in this study. In addition specimens of 11 other species were examined, but since their susceptibilities were not demonstrated they are

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not included in the calculation of the natural incidence of filariasis. In 340 mosquitoes dissected before the 9th day after feeding, the minimum incubation period required for development of the 3rd larval stage of *D. arbuta*, no precociously developed larval stages were found. This would indicate a natural incidence of "no filariasis" in 340 local wild mosquitoes and, by inference, that the 52 per cent infection of my experimental mosquitoes was contracted from the infected porcupine.

Susceptibility of Mosquitoes to D. arbuta Development

Mosquitoes were considered to be susceptible only when the infective (3rd larval) stage was recovered following its feeding on the infected host. The method of the experiment was in itself selective in that the mosquitoes collected were species attacking man. Further selection occurred in the feeding cage for many species would not feed on the porcupine.

In laboratory-reared *Aedes aegypti*, a species foreign to Minnesota, the embryos of *D. arbuta* developed into the infective stage in 10 days. Of 14 mosquitoes which survived the 10-day incubation period 12 yielded the infective stage of the worm.

In *A. canadensis* Theobald the *D. arbuta* embryos developed into the infective stage in 9 days. From all of 15 mosquitoes which survived the incubation period the infective stage was recovered. One mosquito yielded 20 infective stages.

In *A. cinereus* Meigen complete development of *D. arbuta* occurred on the 9th day. Infective stages were recovered from 17 of the 19 *A. cinereus* which survived the incubation period. As many as 29 infective stages were found in one mosquito. From another mosquito 129 first stage larvae were recovered on the 5th day of incubation.

In *A. excrucians* Walker no infective stage larvae were obtained but the recovery from one mosquito of 70 live second stage larvae on the 9th day indicated the probable susceptibility of this species. In another, 121 first stage larvae were found on the 3rd day.

In *A. fitchii* Felt and Young the larvae of *D. arbuta* completed its development on the 10th day of incubation. All of four specimens which survived the incubation period yielded infective stages. From one mosquito 66 second stage larvae were recovered on the 8th day of incubation.

From *A. stimulans* Walker the infective stage was recovered on the 10th day of incubation. All of 8 specimens which lived longer than 10 days yielded infective stages. From one mosquito 28 infective stages were recovered.

Of 28 specimens of *A. vexans* Meigen dissected between the 11th and 61st days after the experimental feeding only one had the infective stage larva although all 28 were infected with less developed forms. In this particular specimen after 29 days of incubation 33 parasites were recovered of which 9 were encapsulated, 4 infective stages (1 encapsulated), 17 second larval stages, and 12 first larval stages (8 encapsulated). In this species a higher incidence of encapsulation of the parasite was associated with resistance to and retardation of its development.

From *Taeniorhynchus perturbans* Walker, out of 9 engorged specimens two were infected, of which one yielded 5 infective stage larvae after 15 days of incubation.

All the above named mosquito species are common throughout the hardwood and coniferous zones of Minnesota.

Pigmental Encapsulation of D. arbuta

Pigmental encapsulation of foreign bodies within the body of mosquitoes has been noted by many observers since Ross (1897) reported black spores in mosquitoes infected with malaria and Noe (1901) reported brown degeneration of *Dirofilaria immitis* in mosquitoes. Yen (1938), who also discussed the nature of these bodies, found encapsulated stages of *Dirofilaria immitis* chiefly in the malpighian tubules, blood content of the mid gut and in the hind gut of mosquitoes. The encapsulated forms of *Dipetalonema arbuta* in the present study were recovered chiefly from the body cavity and the fat body of the mosquito. Of the many observed none were seen which were intimately connected with the tracheal system of the mosquito. Most striking was the recovery of a live infective stage larva enclosed within the pigmented capsule of the second larval stage cuticle. This demonstrated that encapsulation of the living worm occurs. See Pl. II, Fig. 8.

Encapsulation of *D. arbuta* was encountered but rarely in *Aedes aegypti*, *A. canadensis*, *A. cinereus*, *A. excrucians*, and *A. fitchii*. In the last four of these species either microfilarial or first larval stages or both were occasionally found in the encapsulated condition. The encapsulated second larval stage was found in two specimens of *A. aegypti*, in one of *A. cinereus*, and in one of *A. fitchii*. In *A. vexans*, however, all the larval stages including the infective stage were found in the encapsulated condition, and encapsulation of the microfilarial, the first larval, and the second larval stages was common.

Normally the parasite matured to the infective stage within 9 to 11 days in 6 of our mosquito species under laboratory conditions in summer. In *A. vexans*, however, development was retarded, many of the parasites failed to develop, many died, and even after 60 days of incubation some had not developed beyond the first larval stage. The first and second larval stages were found long after the interval normally required for their transition into the next larval stage. The parasite failed to develop at the normal rate in *A. vexans*, and this failure was associated with a more frequent occurrence of encapsulated forms in all stages of development.

Comparison of the Susceptibilities of Mosquitoes to D. arbuta Development

Six local species of mosquitoes in Minnesota and one exotic species which were tested for their susceptibility to infection with *Dipetalonema arbuta* may be grouped as follows according to their fitness as intermediate hosts:

Class I. Efficient and susceptible vectors in which development is normal and larvae usually complete their development.

Group 1. Species in which no encapsulation was observed: *Aedes stimulans* and *Taeniorhynchus perturbans*.

Group 2. Species in which some larvae become encapsulated in the microfilarial (embryonic) stage or in the first larval stage: *Aedes canadensis*.

Group 3. Species in which some larvae become encapsulated in the microfilarial stage, in the first larval stage, or in the second larval stage: *Aedes aegypti*, *A. cinereus*, and *A. fitchii*.

Class II. Inefficient but occasionally susceptible vectors in which development is greatly retarded, larvae complete their development only rarely,

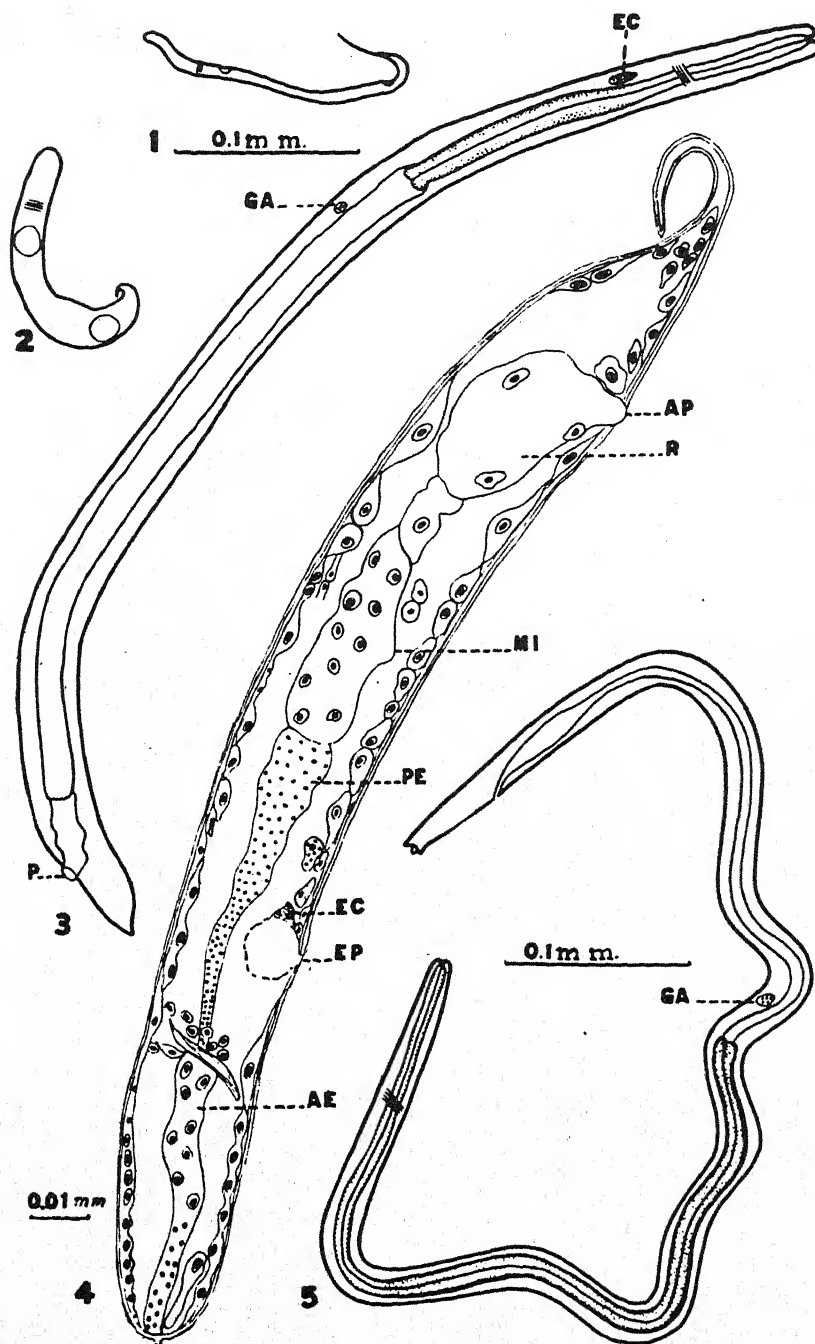


PLATE I

Dipetalonema arbuta Highby, 1943

- FIG. 1. Microfilaria.
 FIG. 2. 1st larval stage.
 FIG. 3. 2nd larval stage.

encapsulation of the first 2 larval stages is common, and encapsulation of the third larval stage occurs: *Aedes vexans*.

Class III. Probable susceptible vector as indicated by normal development up to the third larval stage, third larval stage not demonstrated: *Aedes excrucians*.

Escape of the Infective Larvae from the Labium of the Mosquito

Voluntary emergence of the infective stage of *D. arbuta* was induced by immersion of the labella in fresh warm porcupine serum without pressure, a method used by Blacklock (1926). Emergence was thus induced from all mosquito species tried, *A. aegypti*, *A. canadensis*, *A. cinereus*, and *A. fitchii*. In the first three species named the larvae emerged from the tip of the labella; from *A. fitchii* they emerged through the mesial surface between the tip and base of the labella. By this means as many as 12 worms were observed to make their escape from an infected mosquito in the course of a minute or two.

INCIDENCE OF EXPERIMENTAL MOSQUITO INFECTIONS COMPARED TO FINAL HOST INFECTIONS

No correlation was noted between the rate of infection in the experimental mosquitoes and the degree of parasitism in the final host. One of the most lightly infected porcupines, infected with only one adult female *D. arbuta*, was the source of the greatest rate of mosquito infection, 81 per cent of 57 experimentally fed mosquitoes; while another equally light final host infection was the source of the least rate, 7 per cent of 27 experimental mosquitoes. The intermediate rates of 48 per cent (of 233 mosquitoes) and 70 per cent (of 20 mosquitoes) were from fairly heavy final host infections of 7 and 15 adult female worms respectively.

In this connection it will be noted that Hinman (1935) found no correlation between the number of microfilariae present at any one time in a sample of peripheral blood and the number of adult *Dirofilaria immitis* in the host.

LARVAL DEVELOPMENT OF *Dipetalonema arbuta*

Within two hours after the mosquito had fed upon a porcupine infected with *D. arbuta* the microfilariae migrated into the abdominal cavity and fat body of the mosquito. Masses of them were seen to escape from the confines of the malpighian tubules at their distal tips. A few microfilariae were recovered from the head and thorax of the mosquito during the first 2 days of incubation, but most of them were found in the fat body and abdominal cavity.

The first larval stage was found most commonly in the fat body, frequently in the abdominal cavity, occasionally in the thorax and rarely in the head of the mosquito. It became almost quiescent on the 2nd day, a marked contrast to the sinuous lashing movement of the microfilaria. The former graceful action of the

FIG. 4. 1st larval stage from fresh specimen stained with Azur II.

FIG. 5. 3rd larval stage.

FIGS. 1, 2, 3 and 5 all drawn to same scale.

AE=anterior portion of esophagus

AP=anal pore

EC=excretory cell

EP=excretory pore

GA=Genitalanlage

MI=midintestine

P=anal plug

PE=posterior portion of esophagus

R=rectum

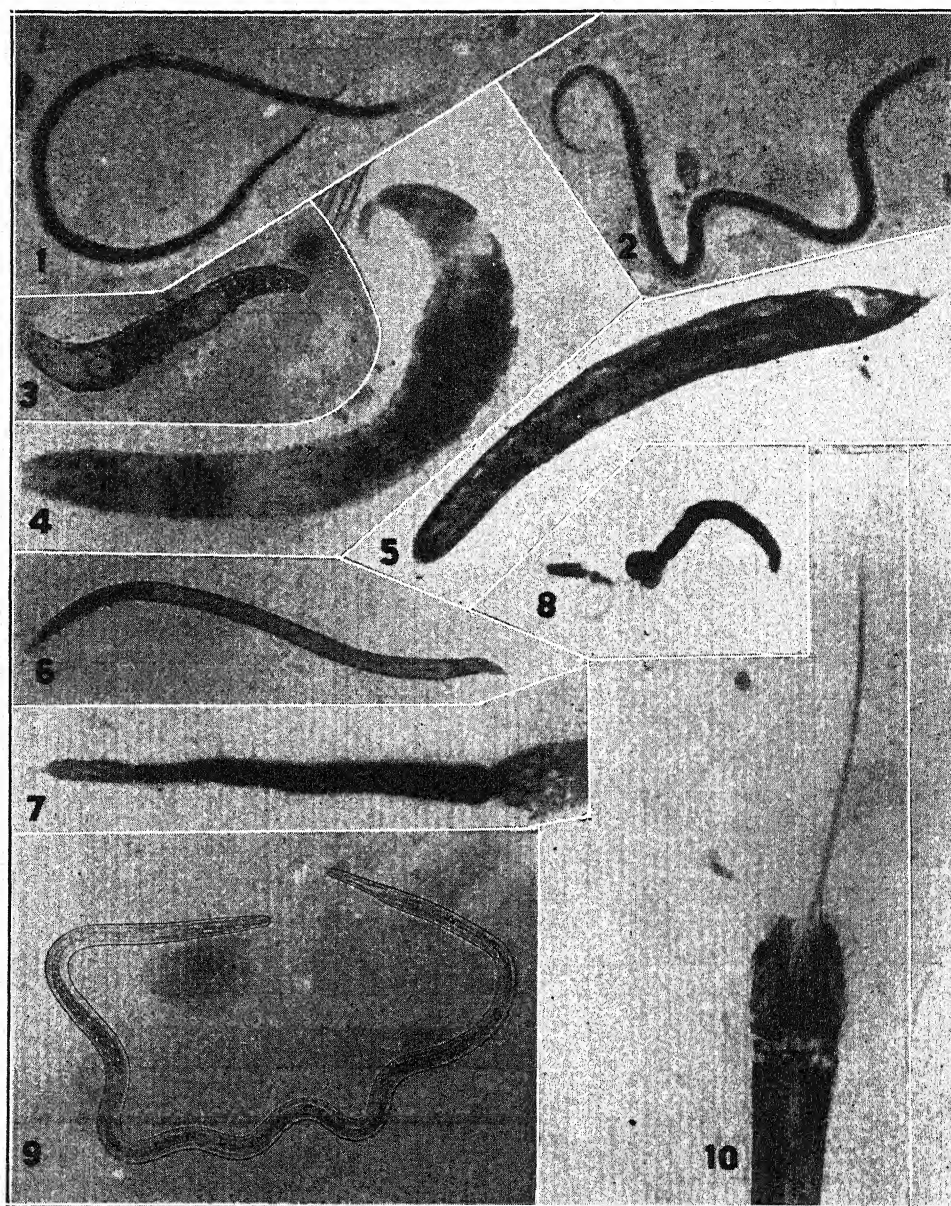


PLATE II

Developmental Stages of *Dipetalonema arbuta*

1. The microfilaria of *D. arbuta*, Wright's blood stain, about $\times 400$.
2. The microfilaria of *Dirofilaria spinosa*, about $\times 400$.
3. The 1st larval stage of *Dipetalonema arbuta* after 5 days in the mosquito, retarded development, beginning of encapsulation, about $\times 333$.
4. The 1st larval stage, 2nd day, normal development, Ehrlich's hematoxylin, about $\times 675$.
5. The 1st larval stage, after about 3 days, normal development, fresh mount, Azur II, about $\times 333$.
6. The 2nd larval stage still within 1st stage cuticle after 8 days normal development, Ehrlich's hematoxylin, about $\times 96$.

worm was first modified by the appearance of a kink in its tail posterior to which the tail seemed to drag passively following the action of the body anterior to it.

The worm became shorter and wider so that by the 3rd day the smallest one noted was 90 μ long by 21 μ wide after fixation in hot Schaudin's fixative. It still retained the cuticle of the microfilaria and the slender posterior portion of the tail was crooked over. In its typical form it resembled a sausage and is called the "sausage stage" by some authors. Grossly it appeared to have two vacuoles and no well defined internal organization when observed in a fresh preparation (Pl. I, Fig. 2). After staining with Azur II, however, details of its anatomy could be seen (Pl. I, Fig. 4). The latter is shown in Pl. II, Fig. 5. Reference may be made to Plate II for photomicrographs of other larval stages.

By the 3rd and 4th days of incubation anlagen of the esophagus, intestine and rectum were clearly delineated, but no lumen had developed. No stoma was present. The esophagus was organized into two parts; its anterior portion was wider than the posterior portion at the junction of the two, but the posterior portion had a greater maximum width and was longer than the anterior portion. The esophagus was about twice as long as the intestine at this stage.

The anlage of the rectum appeared to consist of a syncytium of the 4 rectal cells about 14 μ long. Yamada (1927), Feng (1936), Kobayashi (1940), and Kotcher (1941) found in other filarioids that all 4 rectal cells developed into rectal structures. A structure similar to the anal plug described by Feng (1936) in *Microfilaria malayi* began to be evident in *D. arbuta* on the 3rd day as a rounded elevation at the site of the anal pore of the microfilarial stage. On the 4th day its rounded head was more protruding and proximally a hyaline tapered pointed spike curved anteriorly into the rectum anlage. On the 5th day the head of this anal plug stood out like a button. It persisted through the first and second larval stages.

The nerve ring was at the level of the junction between the two parts of the esophagus. The excretory pore opened into a vesicle of variable size on the posterior wall of which the excretory cell appeared with darkly staining nucleus. The cephalic space was almost obliterated by the third day. The muscle cells enlarged greatly and could be seen in the body wall of the worm.

After the initial shortening to 31 per cent of its original length and increase to 3.3 times its original width the first larval stage increased in size so that by the 5th day of incubation some specimens were 316 μ long by 22 μ wide, or about 1.1 times the length and 3.7 times the width of the microfilaria.

The second larval stage (Pl. I, Fig. 3) developed with or without shedding of the cuticle of the first larval stage. It grew in length and width and became from 553 to 742 μ long and 25 to 29 μ wide, or up to 2.5 times the length and 4.6 times the width of the microfilaria. The anterior extremity was bluntly rounded, and the tail short and conoid in marked contrast to the first larval stage.

The buccal orifice opened into a conoid stoma. The esophagus was 165 to 205 μ long. Its posterior portion had thicker walls and was about twice the length

7. The stoma, esophagus and part of the intestine of the 2nd larval stage, about $\times 300$.

8. The 3rd larval stage within the broken pigmented capsule of the 2nd larval cuticle, about $\times 85$.

9. The 3rd larval stage, fresh preparation, about $\times 120$.

10. The 3rd larval stage fixed in the act of voluntary escape from the labium of the mosquito, after 10 days in the mosquito, about $\times 70$.

of the narrower anterior portion. The lumen of the esophagus communicated with that of the intestine. The sides of the esophagus were slightly constricted where the intestine joined it; this resembled Feng's (1936) description for *Microfilaria malayi* more than that of Kotcher (1941) for *Foleyella* spp.

The intestine was thin-walled and wider than the esophagus. Its lumen frequently contained debris but did not communicate with the rectum. The rectum was about $36\ \mu$ long by $12\ \mu$ wide and was still plugged with the anal plug which could be dislodged from a fresh specimen by pressure on the cover glass. Its structure was continuous with that of the cuticle.

The Genitalanlage appeared as a small mass of cells about $7\ \mu$ in diameter in the body cavity about $43\ \mu$ posterior to the junction of the esophagus and intestine. The excretory cell was located about $30\ \mu$ posterior to the nerve ring at about the level of the junction of the anterior and posterior portions of the esophagus.

This stage was quiescent, almost motionless. It was usually recovered from the fat body of the mosquito. Occasionally specimens were found in the abdominal cavity, and rarely in the thorax and head of the mosquito. It commonly developed from the 5th to the 9th days of incubation.

The third larval stage (Pl. I, Fig. 5) appeared as early as the 9th day of incubation in the mosquito and was recovered alive as late as the 36th day. It was longer and more slender than the second larval stage and very active. Upon being released from the mosquito by dissection it thrashed about vigorously and rapidly crossed the field of the microscope. Within the mosquito's body it migrated freely from one part to another. It was seen to move leisurely down the mosquito's labium, probe at the tip of the labella, retreat back into the head, and a few minutes later it was recovered from the mosquito's abdomen. These larvae were usually recovered from the labium, head, thorax and abdomen of mosquitoes, and rarely from the palps and legs. They quickly escaped voluntarily from the tip of the labella when it was immersed in fresh warm porcine serum. See Pl. II, Fig. 10.

The third larval stage was 0.88 to 1.16 mm long and 16 to $18\ \mu$ wide, 3 to 4 times the length and 2.5 to 3 times the width of the microfilaria. The cuticle was much thicker than that of the earlier larval stages. Its body width was almost uniform except for slight attenuation anterior to the nerve ring and posterior to the anus. The anterior extremity was bluntly rounded and the posterior extremity had 3 papilla-like structures.

The mouth opened at the anterior extremity. The stoma was about $7\ \mu$ long with highly refractive walls. The esophagus was about $553\ \mu$ long, 58 per cent of the worm's length, thus differing from that of *Mf. malayi* which was about 36 per cent according to Feng (1936). The narrower anterior portion of the esophagus was about 20 per cent of the worm's length. The wider posterior portion had cells which stained more deeply with haematoxylin.

The intestine was narrower than the posterior portion of the esophagus. The lumina of the rectum and intestine communicated. The rectum in its mid-region was wider than the intestine, but tapered to the anus, which was about $64\ \mu$ from the posterior extremity. The anal plug of the first and second stage larvae was not present.

The Genitalanlage appeared as a small mass of cells between the intestine and the ventral body wall at a point about $30\ \mu$ posterior to the junction of the esopha-

gus and the intestine. The location of the excretory pore was noted after vital staining of a fresh specimen with methylene blue. Its position was about 50 μ posterior to the nerve ring. The nerve ring was 72 to 86 μ from the anterior end, about 7.5 per cent of the worm's length.

The larval development of *D. arbuta* is fundamentally similar to that described for other filarioid worms. The site of development differs as this is the first of the filarioids reported to develop in the fat body of the mosquito. *Wuchereria bancrofti*, *W. malayi*, and *Foleyella* develop in the thoracic muscles of the mosquito according to Manson (1884), Feng (1936) and Kotcher (1941). *Dirofilaria immitis* and *D. repens* develop in the malpighian tubules of the mosquito according to Grassi and Noe (1900) and Fülleborn (1908). In flies of the genus *Chrysops*, however, development of *Loa loa* in the fat body was reported by Kleine (1915) and Connal and Connal (1922). An interesting comparison of developmental sites is drawn by Chandler et al (1940) with the spiruroid worms, *Habronema* which develop in the fat body and *Draschia* in the malpighian tubules of muscoid fly maggots.

Two moults occur in the development of *D. arbuta* as reported for other filarioids by Yamada (1927), Buckley (1934), Feng (1936), Kobayashi (1940) and Kotcher (1941).

SUMMARY

1. Porcupines from northern Minnesota were found commonly infected with two species of filarioid nematodes, *Dipetalonema arbuta* and *Dirofilaria spinosa*.

2. The microfilariae of these two species could be distinguished from each other by three anatomical characters.

3. The natural incidence of filariasis in selected species of wild mosquitoes from the vicinity of Minneapolis and St. Paul, Minnesota, was none in 340 specimens examined.

4. Six species of Minnesota mosquitoes and one exotic species were susceptible to the complete larval development of *Dipetalonema arbuta*.

5. Marked resistance to the development of the parasite correlated with its pigmented encapsulation was noted in *Aedes vexans*.

6. Pigmented encapsulation was noted to have occurred on a living worm.

7. Voluntary emergence of the infective larvae from the mosquito's labium was observed in 4 mosquito species.

8. No correlation was found between the rate of infection in experimental mosquitoes and the degree of parasitism in the final host source of infection.

9. The site of development was in the fat body, hitherto unreported for filarioids in mosquitoes.

10. The larval development of *D. arbuta* is fundamentally similar to that described for other filarioids.

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VECTORS, TRANSMISSION, DEVELOPMENT, AND INCIDENCE OF
DIROFILARIA SCAPICEPS (LEIDY, 1886) (NEMATODA)
FROM THE SNOWSHOE HARE IN MINNESOTA

PAUL R. HIGHBY

Microfilariae were recovered from the blood contained in the gut of an engorged tick removed from the ear of a snowshoe hare, *Lepus americanus phaeonotus* Allen, which was captured near Baudette, Minnesota. The hare, from which subsequently 8 adult female and 7 adult male *Dirofilaria scapiceps* (Leidy, 1886) were recovered, was subjected to feeding experiments with mosquitoes in order to determine some of the susceptible vectors of the parasite, and to transmit the infection experimentally.

EXPERIMENTALLY SUSCEPTIBLE MOSQUITOES

Captured wild mosquitoes were used in these experiments. Larval filariasis was found in 15 per cent of 139 mosquitoes which were examined by dissection after feeding on the infected hare, and in 15 per cent of 54 mosquitoes examined alive by light transmitted through the labium. That these mosquitoes became infected by feeding on the hare may be inferred by comparison with the natural incidence rate of no filariasis in 340 local wild mosquitoes taken in the same locality (Highby, 1943).

Only those experimentally fed mosquitoes from which the 3rd larval stage of the parasite was recovered by dissection or seen moving within the labium were considered to be susceptible to the complete development of *D. scapiceps*.

In *Aedes canadensis* Theobald the 3rd larval stage was first found on the 12th day of incubation. Of 63 mosquitoes examined after the 11th day of incubation 10 were found to have the 3rd larval stage. Of these, 50 were examined by dissection and 12 by transmitted light through the labium.

In *A. excrucians* Walker the 3rd larval stage was found as early as the 11th day of incubation. It was found in 3 out of 6 mosquitoes which survived the minimum incubation period. Five were examined by dissection and 1 by examination of the labium.

In *A. fitchii* Felt and Young development was completed on the 12th day of incubation. The 3rd larval stage was found in 5 out of 16 mosquitoes examined after the 11th day of incubation. Thirteen were examined by dissection and 3 by examination of the labium.

In *A. vexans* Meigen the 3rd larval stage was found on the 11th day of incubation. Forty-four mosquitoes survived the 11-day incubation period. The 3rd larval stage was found in 1 out of 16 dissected mosquitoes. None were seen by examination of the labium of the remaining 28.

Some evidence of resistance to the development of *D. scapiceps* in *A. canadensis* was noted in the occurrence of pigmental encapsulation of the 1st larval stage and in the occurrence of retarded development as shown by the finding of the 1st larval stage as late as the 14th day and the 2nd larval stage as late as the 13th day of incubation.

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A comparison of the relative susceptibilities of the different species of mosquitoes in this series of experiments is difficult because of the lightness of the infections in the mosquitoes. The heaviest infection encountered in the series of 139 dissections was of 5 infective stage larvae taken from *A. excrucians* on the 11th day.

Mosquito infections were diagnosed as frequently by examination of the labium (15 per cent) as by dissection (15 per cent).

In addition to the 4 species mentioned above which were proved to be susceptible vectors of *D. scapiceps* a 5th species, *A. cinereus* Meigen, was demonstrated to be a transmitter on the basis of a transmission experiment described below.

EXPERIMENTAL TRANSMISSION OF *D. scapiceps* INFECTION TO DOMESTIC RABBITS

Two methods were employed in experiments to infect domestic rabbits with the 3rd larval stage of *D. scapiceps* from the infected mosquitoes; one method was to allow experimentally infected mosquitoes to bite the rabbit and the other was to transfer the larval worm mechanically from the mosquito to the rabbit.

Experimental Transmission by Mosquito Bites

Six domestic rabbits were subjected to infection from the bites of mosquitoes which had fed upon the infected snowshoe hare at least 13 days previously. Blood samples from the experimental rabbits were then examined periodically for the appearance of microfilariae. From 2 of the 6 experimental rabbits microfilariae were recovered.

Experiment with Aedes fitchii Felt and Young

A domestic rabbit was subjected to the bites of 5 specimens of *A. fitchii*, all of which had fed on the infected snowshoe hare not less than 12 days nor more than 17 days previously. (The untimely death of the hare precluded procurement of a greater number of experimentally infected mosquitoes.) These mosquitoes had been examined and in 2 of them the infective stage larvae had been seen moving in the labium. All 5 mosquitoes fed on the experimental rabbit July 18, 1938, and after a 3 day interval fed again, after which they were dissected. From one mosquito 3 infective stage larvae were recovered but the other 4 had no worms. At least one mosquito had been relieved of its infective larvae since 2 were known to have been infected and only one retained infective larvae after the experimental feeding on the prospective final host.

During the ensuing year the peripheral blood of this rabbit was examined periodically for the presence of microfilariae in fresh smear preparations and in centrifuged hemolysed preparations.

A microfilaria was found in a fresh preparation of blood drawn from this rabbit's ear 286 days after it was subjected to infection by the bites of the infected mosquitoes. Thirty days later xenodiagnosis of this rabbit with mosquitoes resulted in the recovery of a microfilaria from the blood content of the mosquito's mid gut. Post mortem examination of the rabbit on the 353rd day yielded no adult worms but a microfilaria was recovered from its lung.

Recovery of 3 microfilariae from this rabbit constitutes evidence of the transmission of *D. scapiceps* from the snowshoe hare to the domestic rabbit by the bites of experimentally infected *A. fitchii*.

Experiment with Aedes cinereus Meigen

Another domestic rabbit was subjected to experimental transmission of *D. scapiceps* infection by the bites of 3 specimens of *A. cinereus* July 19, 1938. These mosquitoes had fed not less than 13 days nor more than 18 days previously upon the infected snowshoe hare. In the course of subsequent periodic examinations a microfilaria was recovered from the peripheral blood of this rabbit 391 days after the experimental exposure to the infection. Upon post mortem examination 30 days later no adult *D. scapiceps* were recovered. The recovery of the microfilaria from the rabbit's blood, however, constitutes evidence of the experimental transmission of *D. scapiceps* from the snowshoe hare to the domestic rabbit by the bites of *A. cinereus*.

Experimental Mechanical Transmission

A domestic rabbit was infected mechanically with 6 infective stage larvae recovered by dissection from 2 mosquitoes which had fed from 11 to 13 days previously on the infected snowshoe hare. These larvae were placed into an abrasion in the skin of the rabbit July 19, 1938. The rabbit was operated upon 240 days later and from its tarsal bursa one adult male *D. scapiceps* was recovered.

Control Animals

A domestic rabbit selected for control died 6 months after initiation of the experiment. Examination for developmental stages and adult worms yielded no specimens.

Four other domestic rabbits which were subjected to experimental transmission by the bites of 4 species of experimentally engorged mosquitoes, were examined for microfilariae and adult worms in the same way as the others but with negative results. The fact that no evidence of transmission materialized in these rabbits would not operate to exclude the mosquito species tried as potential vectors because of the small number of mosquitoes used. These 4 rabbits may, however, be considered as successful controls.

Conclusions Drawn from Transmission Experiments

As a result of these experiments it was demonstrated that the microfilaria of *D. scapiceps* from the infected snowshoe hare can develop in the mosquito to the infective stage, be transferred mechanically to the scarified skin of a domestic rabbit and in the latter host develop to the adult form in 240 days.

It was further demonstrated that *D. scapiceps* infection of the snowshoe hare can be transmitted to domestic rabbits by the bites of *Aedes excrucians* and *A. cinereus* as evidenced by the recovery of microfilariae from the blood of the second definitive host 286 to 391 days after exposure to the infection.

In connection with this experiment 5 control rabbits remained free of the infection.

Discussion of Transmission Experiments

Hitherto only one case of successful controlled experimental transmission of filarioids to new hosts by the bite of infected mosquitoes has been demonstrated. Bancroft (1904) transmitted *Dirofilaria immitis* by infected mosquitoes and recovered adult worms after 7 months from one dog and both microfilaria and adult worms after 9 months from another. Two control dogs remained uninfected for 2 years.

The report of Grassi and Noe (1900) of the recovery of the anterior half of an immature adult *D. immitis* 16 days after mechanical inoculation of a dog with infective larvae from a mosquito was severely criticized by Annett, Dutton and Elliott (1901). Noe's (1900) uncontrolled effort at transmission of *D. immitis* by mosquito bite appeared to be very unsatisfactory to Sambon (1902). The dog was not selected and the mosquitoes were not subjected to controlled feeding upon a source of infection.

Fülleborn (1929) reported transmission of *D. immitis* by mechanical inoculation with recovery of microfilariae after 8 months from 4 out of 5 dogs and adult female worms from the 5th dog.

Failure attended the efforts of Hinman (1935) to transmit *D. immitis* through *Aedes aegypti*. Connal and Connal (1922) failed to transmit *Loa loa* to laboratory animals. No further report has come from Poynton and Hodgkin (1939) regarding their attempt to obtain the adult of *Mf. malayi* by transmission from man to monkey.

Yokogawa (1939) reported an infection experiment with *Wuchereria bancrofti* on 5 volunteers. In one case 82 days after infection, and in another 111 days after the first and 56 days after a second infection, 2 microfilariae were recovered from 2.0 ml of blood examined at night. He believed that larvae may become adult and produce microfilariae 20 to 30 days after infection. It would seem that an account should have been given of precautions taken to avoid infection of the volunteers previous to the experiment.

NOTES ON THE LARVAL DEVELOPMENT OF *D. scapiceps*

The developmental stages of *D. scapiceps* in the mosquito are much similar to those of *Dipetalonema arbuta* and of other filarioids which have been reported. Within 9 hours after ingestion the microfilariae were found exsheathed in the body cavity. Escape from the microfilarial sheath may occur in the body cavity. One was recovered from the body cavity half-way free of its sheath and entangled with the mosquito's tracheal system during the 1st day of incubation. In this respect the development of *D. scapiceps* would differ from that of *Wuchereria bancrofti* which escapes from its sheath in the mid gut according to Manson (1884), Yamada (1927), and Kobayashi (1940).

The 1st and 2nd larval stages were recovered from the fat body of the mosquito.

The 3rd larval stage was 780 μ long by 14 μ wide. Three blunt papillae were present at its posterior extremity. Its earliest appearance was on the 11th day of incubation in the mosquito. It was recovered from the labium, head, thorax, body cavity and fat body.

THE MICROFILARIA OF *D. scapiceps*

Microfilariae in rabbits from North America have been reported 3 times previously. The occurrence of microfilariae in the blood of a rabbit from British Columbia was reported by Harkin (1927). Schwartz and Alicata (1931) described microfilariae from the blood of *Lepus washingtonii* from the state of Washington, and conjectured the probability of their being the larvae of *D. scapiceps*. In *L. americanus* from Ontario microfilariae were reported by MacLulich (1937).

In connection with this investigation I studied specimens of microfilariae obtained from the lymph at the site of infection, the tarsal bursa of a snowshoe hare

infected with 8 female and 7 male adult *D. scapiceps*. The specimens were not allowed to dry but were studied in fresh preparations of aqueous Azur II.

Microfilaria of *Dirofilaria scapiceps*

(Fig. 1)

Microfilaria sheathed, finely striated transversely. Length 205 to 270 μ (243 μ).* Width 6 μ . Anterior end with protrusible point, bluntly rounded when retracted. Tail attenuated with blunt tip. Cephalic space 3 μ long. First 4 nuclei of nuclear column present scattered appearance. Position of anatomical points in terms of distance from anterior extremity expressed in percentage

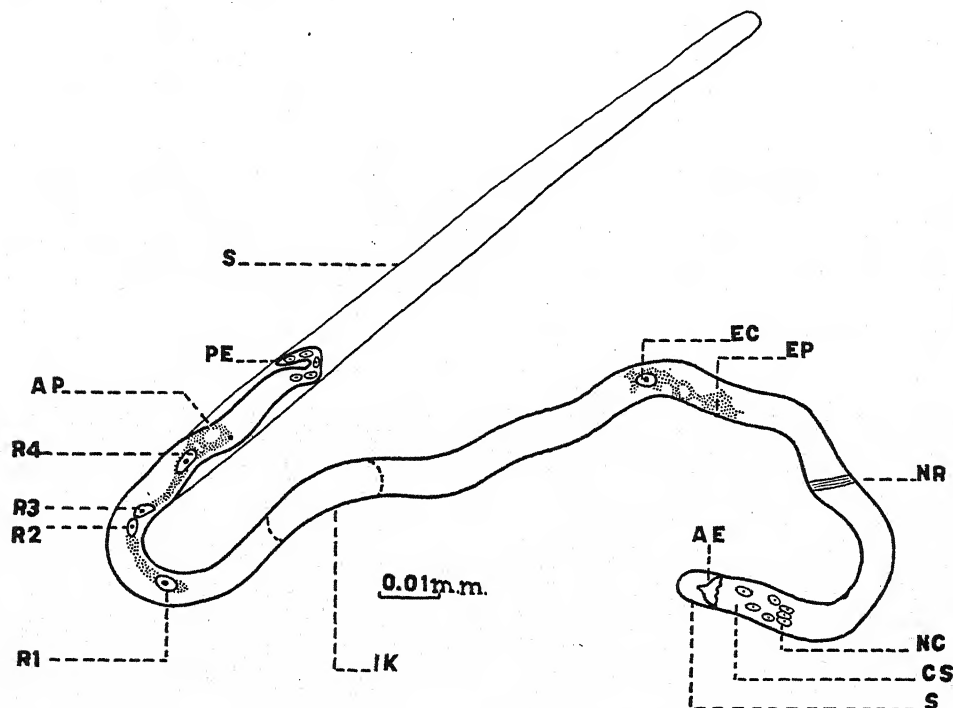


FIG. 1. Microfilaria of *Dirofilaria scapiceps* (Leidy, 1886).

- | | |
|----------------------------|---------------------------------------------------------------------------------------------------------------|
| AE—anterior extremity | NC—anterior elements of nuclear column (only a few of the most anterior and most posterior nuclei are shown). |
| AP—anal pore | NR—nerve ring |
| EC—excretory cell | PE—posterior extremity |
| EP—excretory pore | R ₁ , R ₂ , R ₃ , R ₄ —1st, 2nd, 3rd, and 4th rectal cells |
| CS—cephalic space | S—sheath |
| IK—"Innen Körper" location | |

of the worm's entire length: nerve ring 20.8 to 24.5 (22.7); excretory pore 31.2 to 36.6 (33.2); excretory cell 36.8 to 42.7 (39.2); "Innen Körper" anterior limit 51.6 to 61.2 (55.2), posterior limit 56.9 to 68.8 (63.4), length 3.1 to 17.1 (8.9); 1st rectal cell 68.7 to 75.6 (72.2); and anal pore 82.0 to 86.0 (84.1). Last 4 to 7 nuclei at posterior end of nuclear column arranged in linear series, and become progressively more attenuated toward posterior end. Last 3 nuclei scattered with clear spaces between them. Last nucleus more rounded, located at extreme tip of tail and gives tail tip blunt appearance.

A comparison of the description given above with that given for *Mf.* species (? *Dirofilaria scapiceps*) by Schwartz and Alicata (1931) reveals certain differences

* Figures in parentheses denote average of 20 specimens measured in aqueous Azur II; except "Innen Körper," average of 9 specimens.

as well as similarities. Perhaps the differences might appear less pronounced if material from the 2 sources, *L. americanus phaeonotus* and *L. washingtonii*, were prepared for study by similar treatment. The writer cannot determine these larvae as being specifically identical from objective consideration of the material at hand.

INCIDENCE AND DISTRIBUTION OF *D. scapiceps* INFECTION
IN SNOWSHOE HARES IN MINNESOTA

Snowshoe hares from 3 widely separated sections representing the eastern, northwestern, and southwestern extremes of the range of the hare in Minnesota were examined in 1938-1939. From the Lake Superior watershed in Lake County 18 infections were found in 31 hares (58 per cent). From Lake of the Woods County 2 infections were found in 15 hares (13 per cent). From this region Manweiler (1938) reported infection of "approximately one-third of all specimens of snowshoe hares examined" in the winters of 1936-37 and 1937-38. From Morrison County in central Minnesota 3 infections were found in 11 hares (27 per cent). This sampling indicates that the infection was common throughout the range of the snowshoe hare in Minnesota in 1938-39.

A total of 57 hares were examined and 23 cases of infection were found. From these infections 176 adult *D. scapiceps* were recovered of which 106 were females and 70 males. In addition there were 8 cases of calcification of the worms all of which developed in hares from the Lake County region. No worms were found in the fore feet, subcutaneously in the lumbar region, or in the intermuscular fascia of the large muscles of the leg, shoulder, and back. Except for one case the parasites were all found in the tarsal bursa, a pocket below the distal end of the tibia on the anterior aspect of the tarsal joint covered with tough connective tissue. The exception noted was one recovered from under connective tissue on the posterior aspect of the tarsal joint. The heaviest infection was 18 worms, 9 males and 9 females in one case, and 6 males and 12 females in another.

SUMMARY

1. Five species of Minnesota mosquitoes were demonstrated to be susceptible to the complete larval development of *Dirofilaria scapiceps*. The susceptible species are: *Aedes canadensis*, *A. cinereus*, *A. excrucians*, *A. fitchii* and *A. vexans*.

2. Transmission of *D. scapiceps* from the snowshoe hare to the domestic rabbit was accomplished by mechanical transfer of the infective stage larvae from experimentally infected mosquitoes to the scarified skin of the rabbit, and resulted in the recovery of an adult *D. scapiceps* from the experimental rabbit 240 days later.

3. Transmission of *D. scapiceps* from the snowshoe hare to domestic rabbits by the bite of experimentally infected mosquitoes was accomplished through *A. fitchii* and *A. cinereus*.

4. Microfilariae were demonstrated in the blood of 2 experimentally infected domestic rabbits 286 to 391 days after exposure to infection.

5. The transmission experiments were controlled by 5 rabbits which remained free of the infection.

6. Larval filariasis in mosquitoes may be detected as frequently by examination of the labium of the living mosquito as by the method of dissection.

7. The larval stages of *D. scapiceps* were grossly similar to those of other filarioids.
8. The microfilaria may escape from its sheath while in the body cavity of the mosquito.
9. The 1st and 2nd larval stages were found in the fat body of the mosquito.
10. The earliest noted appearance of the 3rd larval stage was on the 11th day of incubation in the mosquito.
11. The microfilaria of *D. scapiceps* is described and figured.
12. *D. scapiceps* infection of the snowshoe hare is common throughout the range of the hare in Minnesota as shown by incidence rates of 13 to 58 per cent in samples from 3 geographic extremes of its range.
13. Xenodiagnosis of filariasis by ticks and mosquitoes was useful.

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BLOOD PROTOZOA OF BIRDS TRAPPED AT ATHENS, GEORGIA*

HELEN B. JORDAN

A considerable amount of information concerning the incidence of blood parasites in wild birds in certain sections of the United States has been presented by various investigators. Among these Huff (1939) reported on smears from 967 birds from widely different areas, Manwell and Herman (1935) examined 652 birds at Syracuse, New York, and Cape Cod, Massachusetts, and Wetmore (1941) 618 birds in the District of Columbia and Patuxent Refuge vicinity. Less extensive surveys were carried out by Coatney and Jellison (1940) on 20 birds taken in Beaverhead County, Montana, by Coatney and West (1938) on 84 birds in Nebraska, by Herns et al. on 150 birds in California, and by Paul E. Thompson on 275 birds trapped in Bulloch and Clarke Counties, Georgia (1943).

The author, during the last two years, has examined blood smears from 1103 birds trapped and banded on the campus of the University of Georgia at Athens. Smears from these birds were stained with MacNeal's tetrachrome and examined with 15X oculars and 1.8 mm oil immersion objective for at least ten minutes in each case.

Table 1 gives the incidence of *Haemoproteus*, *Plasmodium* sp. and *Trypanosoma* found.

TABLE 1.—Distribution of blood protozoa in birds examined at Athens, Georgia

Host		Parasite										
Scientific name	Common name	Negative	<i>Plasmodium</i> sp.	<i>P. relictum</i>	<i>P. cathemerium</i>	<i>P. elongatum</i>	<i>P. hexamerium</i>	<i>P. vaughani</i>	Total <i>Plasmodium</i>	<i>Haemoproteus</i>	<i>Trypanosoma</i>	Total birds
<i>Passer domesticus</i>	English sparrow	418	5	42	14	9	70	..	1	489
<i>Toxostoma rufum</i>	Brown thrasher	111	11	2	4	22	2	..	41	63	..	215*
<i>Cyanocitta cristata</i>	Blue jay	56	24	..	80
<i>Zonotrichia albicollis</i>	White throated sparrow	78	32	1	110*
<i>Mimus polyglottos</i>	Mocking bird	29	3	2	..	1	6	31	..	66
<i>Pipilo erythrophthalmus</i>	Towhee	13	8	2	5	..	15	12	1	40*
<i>Richmondia cardinalis</i>	Cardinal	7	4	8	1	5	18	7	2	25*
<i>Sturnus vulgaris</i>	Starling	16	16
<i>Quiscalus quiscula</i>	Purple grackle	1	..	1	1	..	2	1	..	3*
<i>Spizella passerina</i>	Chipping sparrow	22	11	..	33
<i>Turdus migratorius</i>	Robin	2	1	..	1	1	3	4*
<i>Dumetella carolinensis</i>	Catbird	5	1	1	2	7
<i>Spizella pusilla</i>	Field sparrow	1	1	1	..	1	3	3*
Total		759	1091

* Double infections.

In addition to birds listed in Table 1, two humming birds, one red-headed woodpecker, one loggerhead shrike, one redstart, one prairie warbler, one hairy woodpecker, one domestic pigeon, and one gray-cheeked thrush were all negative; one

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flicker, and one blue bird showed an infection with *Haemoproteus*, and one wood-thrush *P. cathemerium*.

These studies appear to include 13 new host records for *Plasmodium* and 1 for *Haemoproteus* not present in the check-lists of Coatney (1936), Coatney and Roudabush (1936), and the above mentioned surveys, as follows: *Plasmodium elongatum* Huff, *P. hexamerium* Huff, *P. relictum* Grassi and Feletti, and *P. cathemerium* Hartman, in the brown thrasher, *Toxostoma rufum*; *P. relictum* and *P. elongatum* in the mocking bird, *Mimus polyglottos*; *P. relictum*, *P. hexamerium*, and *Haemoproteus* in the towhee, *Pipilo erythrophthalmus*; *P. cathemerium* in the cardinal, *Richmondia cardinalis*; and *P. cathemerium* and *P. hexamerium* in the robin, *Turdus migratorius*; *P. relictum* in the catbird, *Dumetella carolinensis*; *P. hexamerium* in the purple grackle, *Quiscalus quiscula*; and *P. elongatum* and *P. relictum* in the field sparrow, *Spizella pusilla*.

In most instances the number of birds of a single species taken within a limited period was too small to make possible an adequate picture of the incidence of parasites for that particular host by seasons. In the case of *Plasmodium* in English sparrows, *Haemoproteus* in jays, and *Plasmodium* and *Haemoproteus* in brown thrashers, however, enough individuals were taken to make a few comparisons. These observations are recorded in Table 2 and graphically shown in Fig. 1.

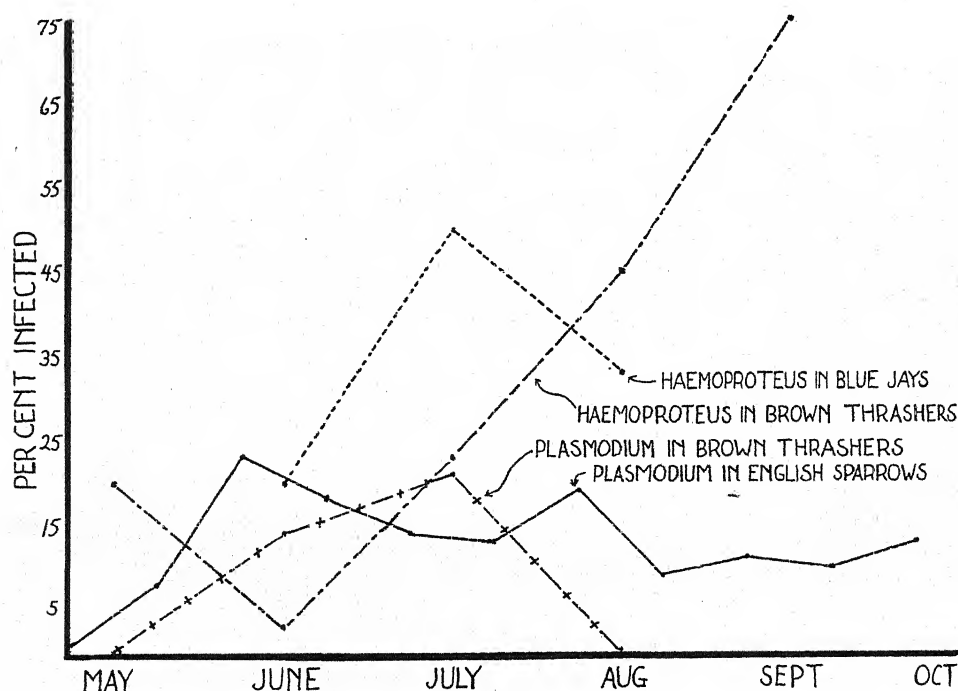


FIG. 1. Graph showing the incidence of *Plasmodium* and *Haemoproteus* in blue jays and brown thrashers from May through September, and of *Plasmodium* in the English sparrow from May through October.

These figures show that there are rather abrupt changes in incidence from month to month which make comparisons of incidence in different regions meaningless unless one also has information on the dates of examination.

Some of the infections were diagnosed on the basis of examination of one slide only, and some of the infections were diagnosed on the basis of examinations of several slides made at different times of day over a period of two or three days. Subinoculation into canaries was carried out in the following instances: *P. hexamerium* from the towhee, *P. relictum* from the cardinal, towhee, and purple grackle; *P. cathemerium* from the robin and towhee. One of the *P. relictum* infections in an English sparrow was observed at intervals for three days and found to show the matinal schizogony described by Huff (1937) in the case of a strain of *P. relictum* isolated from a robin.

TABLE 2

Host and parasite	May		June*		July		August	
	No.	%	No.	%	No.	%	No.	%
English sparrow <i>Plasmodium</i> ...	71	1	44	19	93	16	113	16
Brown thrasher <i>Plasmodium</i> ...	5	0	36	14	38	21	27	0
Brown thrasher <i>Haemoproteus</i> ..	5	20	36	3	38	23	27	45
Blue jay <i>Haemoproteus</i>	†	..	10	20	28	50	15	33

* During the first two weeks of June the infection reached a peak of 23%.

† No jays examined in May, but 16 caught in February, March, and April were all negative.

Plasmodium relictum seems to be not only the most widely distributed species of the avian malaria parasite but also the most abundant. Of all the plasmodia found in birds examined in Athens from March through August, 1940, and from March through October, 1941, 37% were *P. relictum*; 24% *P. elongatum*; 13% *P. cathemerium*; and 6% *P. hexamerium*; 21% were of undetermined species. Only one infection of *P. vaughani* was found. This was in a young robin.

Table 1 shows that of the 12 recognized species of avian malaria parasites *P. relictum*, *P. elongatum*, *P. cathemerium*, *P. hexamerium*, and *P. vaughani* were found in birds at Athens, Georgia. Other investigators have reported *P. nucleophilum*, *P. circumflexum*, *P. polare*, and *P. oti* from the United States. That our survey includes none of these plasmodia may be a result of our inclusion of very few or none of the host from which these species were recorded.

About 1 out of 7 birds showed infection with plasmodia during the season covered in our survey. Many of them showed double infections. Two triple infections in the case of young female cardinals were noted. Double infections were encountered as follows:

English sparrow	<i>P. relictum</i> and <i>P. elongatum</i> ; <i>P. relictum</i> and <i>P. cathemerium</i> .
Brown thrasher	<i>P. elongatum</i> and <i>Haemoproteus</i> .
White throated sparrow	<i>Haemoproteus</i> and <i>Trypanosoma</i> .
Mocking bird	<i>Haemoproteus</i> and <i>P. relictum</i> .
Towhee	<i>Haemoproteus</i> and <i>Trypanosoma</i> ; <i>P. hexamerium</i> and <i>P. relictum</i> .
Cardinal	<i>P. cathemerium</i> and <i>Haemoproteus</i> ; <i>P. cathemerium</i> , <i>P. relictum</i> , and <i>P. elongatum</i> ; <i>Trypanosoma</i> , <i>P. relictum</i> , and <i>Haemoproteus</i> ; <i>P. relictum</i> and <i>P.</i>

elongatum; *P. relictum* and *Haemoproteus*; and *P. relictum* and *Trypanosoma*.
 Purple grackle *Haemoproteus*, *P. hexamerium*, and *P. relictum*.

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OVERWINTER LOSS OF NODULAR WORM LARVAE FROM A SHEEP PASTURE AND ITS BEARING ON THE CONTROL OF NODULAR WORM DISEASE¹

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In the development of methods for the control and eradication of ruminant parasites the importance of directing attention to the free-living stages which occur on pastures as well as to the parasitic stages which develop within the host has been repeatedly emphasized. One of the methods advocated for ridding pastures of the free-living stages of parasitic worms has been the "resting" of pastures for a number of months or over winter to allow the free-living stages to die off. Existing information as to the value of this method in the control of particular species of parasites is insufficient to formulate a sound control program for sheep parasites as a whole. The present report concerns an experiment devised to test the value of resting pastures overwinter as a measure for controlling nodular worms in sheep.

REVIEW OF WORK OF PREVIOUS INVESTIGATORS

In Louisiana, Dalrymple (1904) observed that a pasture on which 2 lambs became infected in August did not contain infective larvae the following June, since 3 lambs which grazed on it at that time failed to become infected. In this case the survival test was complicated by the fact that the pasture had been plowed, seeded, and a crop harvested during the interval.

Ransom (1906) noted the failure of nodular worm larvae to survive overwinter from October to June on pastures on high ground and on low, swampy ground in Maryland.

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¹ The experiment reported in this paper was carried out at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

Smith and Ring (1927) found that a clean flock of sheep did not acquire nodular worm infection when put on a previously infested swampy pasture in New Jersey which had been rested from November of one year to May of the second year.

Rebrassier (1933) gave an abstract of an experiment in which nodular worm larvae survived at least one winter on a pasture in Ohio, as determined by placing susceptible lambs on it.

Baker (1938, 1939) listed this parasite among the sheep nematodes which survived overwinter from July to the following June on a hillside pasture in New York. It is impossible to tell from his published data whether or not the nodular worm was one of the "strongyles" which he reported in a second experiment to have survived "at least 21 months."

In Canada, Swales (1940) found that nodular worm larvae died out overwinter on pastures, as shown by failure of spring lambs to become infected in June on pastures known to have been contaminated the previous autumn.

Shorb (1942) found that nodular worm larvae failed to survive on experimental pasture plots at Beltsville, Maryland, for more than $2\frac{1}{2}$ months in summer, or $3\frac{1}{2}$ months in summer and fall, and that the larvae of this parasite did not apparently develop on pastures in the late fall, winter and spring.

MATERIALS AND METHODS

1. *The experimental pasture.*—The pasture used was 0.7 acre in size and was separated on two sides from the adjacent, unoccupied paddocks by a high crowned road and on the other two sides by a ditch between a double fence. The pasture was shaded in spots by 46 scattered trees—mostly young chestnut-oak and red oak. The pasture sloped gently toward the northeast. Situations regarded as especially favorable for the development and survival of nematode larvae existed in the shade on two sides of the pasture; in the masses of fecal pellets which accumulated during the contamination period at the edge of bare areas under the trees that were frequented by the sheep; and in the shade of the A-type shelter where feces accumulated at first in the grass and later on the bare, packed ground. The soil was of a poor quality, heavy sandy loam to almost a clay loam type. The forage was relatively sparse and consisted of mixed pasture grasses, clovers, and weeds. Special precautions were taken to guard against accidental contamination, from any extraneous source, of the pasture, feed, and water. Only two persons entered the pasture, and only after putting on carefully scrubbed and rinsed rubbers.

2. *Experimental animals.*—All the sheep used in the experiment had been raised in stalls until weaning time, when they were put into raised, concrete-floored cages outdoors. Both stalls and cages were carefully cleaned and allowed to dry thoroughly before the lambs were placed in them. Bedding was removed with the accumulated feces three times each week. Although the ewes from which the experimental lambs were raised were not free from worms, this method of raising lambs, with only rare exceptions, proved at this station to be satisfactory for the prevention of all parasitic infection, except the almost ubiquitous coccidia and *Strongyloides*. Special precautions were taken to prevent accidental infection of the 6 test lambs. Chance of infection passing from ewes to lambs before weaning time was minimized because the ewes had been treated with phenothiazine the previous fall and again just before parturition, and also because the lambs were weaned when they were 7 to 8 weeks

old. The cage into which the lambs were put had been carefully scrubbed with chloride of lime.

3. *Evidence of freedom of the test lambs from accidental infection.*—The first fecal examination, made on the 6 test lambs at the time they were weaned, revealed numerous coccidial oöcysts and small numbers of *Strongyloides* eggs. A second examination 26 days later gave similar results, cultures of 1 to 2 grams of feces from each lamb yielding only small numbers of *Strongyloides* larvae. Even though no other species of worms were found, it was decided a week later to reduce still further the chance of an accidental infection by treating each of the 6 lambs with 8 to 10 grams of phenothiazine. A third fecal examination was made a week later, long enough after weaning (38 days) for the development of any of the common nematodes occurring in sheep, if infection had been acquired while the lambs were with the ewes. In addition to the two types of parasites found previously, one *Nematodirus* egg was found in the droppings of lamb C. This showed that despite all precautions a light *Nematodirus* infection had been acquired by one of the lambs and suggested the possibility that the other lambs possibly also harbored subdetectable infections with the same species. A second test made at the same time (3 days before four of the lambs were put on pasture) by culturing 1 to 6 grams of feces from each animal, again yielded only *Strongyloides* larvae.

4. *Methods.*—Fecal examinations were made by microscopic examination of salt flotation preparations representing one-half gram of feces.

At post-mortem examination the fourth stomach, small intestine, and large intestine were carefully searched for worms and worm lesions. Each part of the alimentary canal was then stripped separately, the contents screened through screens of coarse, medium and fine mesh, and the screenings searched carefully for worms.

The number of eggs passed per day by the sheep which contaminated the pasture was calculated from the eggs-per-gram counts and the monthly weights of the sheep, using the conversion factors established by Stoll (1929) for the appropriate fecal consistency.

5. *Weather data.*—Weather data for the three periods of the experiment are given in Table 1. These local data include figures for the monthly average, and range, of the maximum and minimum daily temperature readings made at the laboratory of the Zoological Division at the Beltsville Research Center by Mr. A. Dinaburg. The monthly rainfall was totalled from the record of daily rainfall made by the Bureau of Dairy Industry at a point approximately 1.2 miles from the experimental pasture. The conclusions drawn as to the normality of the weather conditions during the three periods of the experiment are based on the deviations from normal listed in the monthly meteorological summaries of the U. S. Weather Bureau for Washington, D. C., which is approximately 15 miles away.

PLAN OF EXPERIMENT AND RESULTS

I. Contamination of the Pasture

The pasture used in the experiment had been exposed to daily contamination by nodular worm eggs passed in the feces of from one to four sheep between May 10 and November 1, 1940. One of these sheep was a yearling which had been experimentally infected with nodular worms 3 months previously, the infective larvae having been cultured from eggs taken from adult worms. This sheep was passing

approximately 100,000 eggs each day when put on the pasture. The other three animals were spring lambs which picked up nodular worms from the pasture. One began to pass eggs at the end of July and the other two at the end of August. During the 6 months' period the 4 sheep passed a calculated total of 89,500,000 eggs onto the 0.7 acre pasture, and all showed, by gradual increase in the number of eggs passed, evidence of reinfection. All four sheep also passed numbers of *Strongyloides* eggs and coccidial oöcysts during the entire period, and in August very small numbers of *Trichuris* eggs were observed in the feces of two of the sheep, and *Capillaria* eggs in the feces of one.

Weather conditions were fairly normal during this period except that in June it was unusually hot and the rainfall markedly subnormal (Table 1). Rain fell on

TABLE 1.—Local weather data (Beltsville, Md.) for the three periods of the experiment

Experimental period	Date	Air temperature (degrees F.)				Precipitation	
		Maximum		Minimum		Total inches	Number of days on which rain fell
		Range	Average	Range	Average		
I. Period of contamination	1940						
	May	51-91	76	38-61	51	3.8	8
	June	72-91	83	44-69	61	0.6	2
	July	74-100	87	49-84	64	3.1	6
	Aug.	68-92	82	53-72	63	3.0	11
	Sept.	64-90	77	36-67	52	4.3	7
	Oct.	45-80	63	26-53	42	3.3	6
II. Overwinter rest period	Nov.	37-70	53	21-46	34	4.9	7
	Dec.	28-64	48	6-46	27	2.3	8
	1941						
	Jan.	32-53	39	10-33	21	3.1	7
	Feb.	30-52	41	11-34	21	0.9	4
	Mar.	25-62	48	12-44	25	1.7	5
	Apr.	56-94	72	28-57	43	2.7	4
III. Period of pasture test	May	59-95	81	36-69	51	1.3	11
	June (2nd through 15th)	64-87	68	46-62	51	3.7	9

at least 40 separate days and amounted to a total of 18.1 inches, which was 4.5 inches less than normal. Conditions for the development and survival of larvae were considered good in May, poor in June, relatively poor in July and August because of depleted forage and a preponderance of hot, dry days, probably quite favorable in September, and unfavorable in October on account of the decreasing temperature. During the last three months of the period of contamination only scattered shoots of grass were left for the sheep to graze on.

II. Overwinter Resting of the Pasture

The pasture was unoccupied and unentered during the 7-month period from November 1, 1940 to June 2, 1941. The weather (Table 1) was fairly normal during the winter, although the amount of snow was slightly less than usual. The temperature was slightly subnormal in February and March, and slightly above normal in April and May. From February through May there occurred a dry spell with subnormal humidity and increased hours of sunshine, and a cumulative deficiency in rainfall. However, rain fell on at least 46 days in the 7-months' period, although the total amount (16.9 inches) was 6.3 inches less than normal. Fecal pellets remained intact on the pasture throughout this period, and, until a new

growth of grass appeared late in March, the pellets were exposed to the action of sun, wind, and rain, except in the shelter and in a few places along the fences where the pellets were covered by leaves. By the end of the period a good growth of short grass had appeared again and hidden the pellets.

III. Test for Survival of Larvae on Pasture

A. *Direct test for presence of eggs and larvae.*—At the end of the rest period, namely on June 2, 1941, an attempt was made to recover nodular worm eggs from the ridges of pellets found at the edges of the bare areas around trees, and from the pellets and soil. Pellets were soaked in water, crushed and examined by the usual flotation method; a few sporulated and apparently viable coccidial oöcysts were found, but nematode eggs were absent. A 19-gram sample of pellets and underlying topsoil was placed in a Bearmann funnel; only free-living nematodes were recovered.

B. *Animal test for survival of larvae.*—On June 2, 1941, two of the test lambs (A and B) were placed on the experimental nodular worm pasture and left there for two weeks with the grass as their only source of food. This interval was considered long enough to allow extensive grazing over the entire pasture and yet short enough to eliminate practically any possible chance of the lambs reinfecting themselves as the result of infection picked up on the pasture or of the presence of sub-detectable infections in the lambs. Another pair of lambs (C and D) were left in the cage as controls to test the possibility of accidental acquisition of infection from ewes, stalls, or cage. The third pair of lambs (E and F), which served, when slaughtered, as additional controls on the freedom of the test lambs from accidental infection with nodular worms, were placed for the same interval on a pasture which had been contaminated the previous summer with the sheep stomach worm, *Haemonchus contortus*. The result of this test will be reported in detail elsewhere (Sarles, 1943).

Weather conditions were considered exceptionally favorable for the acquisition of infection while the lambs were on pasture (Table 1). The lambs went on pasture at the end of three days of rain which had followed two weeks of rainless weather. Consequently the ground, the fecal pellets still present from the previous year, and the grass were all soaking wet when the lambs first came in contact with them, and if any infective larvae had been present in the soil or pellets they should have had optimum stimulation to migrate from the soil or pellets onto the grass. Moreover, the weather continued to be abnormally wet, alternating between periods of several days of cloudy, wet, humid weather, and occasional days of clear weather with bright sunshine. Rain fell on 9 of the 14 days, and totalled 3.7 inches; sometimes it fell in the form of heavy showers and sometimes as a slow drizzling rain. Direct observation of the forage and of the behavior of the sheep revealed that the latter grazed in all kinds of weather, even during showers, over the entire pasture, both on short and long grass as well as on weeds.

The two pairs of lambs (A and B, and E and F) were taken off pasture at the end of two weeks and returned to the cage containing the third pair. A third fecal examination made at this time, 55 days after the lambs had been weaned, revealed in the feces of all 6 lambs only the previously detected coccidial and *Strongyloides* infections.

The final fecal examination, made after the lambs had been held in the cage for 3 more weeks to allow time for development to macroscopic size of any worms which might have been picked up from the pasture, gave the same negative result, except for one *Trichuris* egg from lamb C, the same unpastured animal which had previously shown a *Nematodirus* egg on one examination.

Lambs A and B were slaughtered 22 and 25 days, respectively, after removal from the nodular worm contaminated pasture. No nodular worms, and no nodules or other worm lesions, were found in the intestines of either lamb, and the fourth stomach of each was also free from worms. One *Nematodirus* was recovered from the small intestine of lamb A, and 3 *Nematodirus* and 8 *Strongyloides* from lamb B.

Lambs E and F were slaughtered 23 and 24 days, respectively, after removal from the stomach worm contaminated pasture. Neither showed worm lesions, stomach worms, or nodular worms, but one *Nematodirus* and 33 *Strongyloides* were recovered from lamb E, and 67 *Strongyloides* from lamb F.

Since the only species of worms found in the test sheep were small numbers of two of the three species of nematodes already known to be present in some of the test lambs as the result of accidental infection, the experiment showed that nodular worm larvae had not survived overwinter on the pasture. Because the result of the experiment was negative, and because the two pairs of pastured lambs served as controls on each other, it was considered unnecessary to slaughter the third pair of lambs at this time. Lamb C was slaughtered a year later, after having been held in the meanwhile in a cage to preclude accidental infection. At necropsy it was found to harbor 2 *Trichuris* and one *Nematodirus*. Lamb D was not killed, but during the year that elapsed before it was used for another experiment only *Strongyloides* eggs were seen in repeated fecal examinations.

DISCUSSION

Nodular worm larvae did not survive overwinter on a pasture in Maryland under the conditions of this experiment. This result confirms the findings of Dalrymple (1904), Ransom (1906), and Swales (1940) and gives added support to the preponderance of evidence showing that the free-living stages of *O. columbianum* are not highly resistant or able to survive overwinter in northern climates.

As Swales (1940) and Gordon (1941) have already emphasized, the use of this naturally occurring overwinter sterilization of sheep pastures in conjunction with medicinal treatment has great possibilities in controlling nodular worm disease in sheep. Contaminated pastures should be rested overwinter during the period when they are of little use for grazing, to make them safe for uninfected lambs and for breeding stock which have been treated. Inasmuch as the larvae do not survive overwinter on pasture it follows that breeding stock must carry the infection over from one year to the next, and these sheep constitute, therefore, the real source of danger to spring lambs and to themselves by initiating new pasture contamination. It is, therefore, very important in the control of this debilitating infection to attempt to eliminate all residual infection from breeding animals during the winter and/or early spring by anthelmintic treatment, and then to place these animals on pastures which have not been contaminated since the last grazing season.

SUMMARY AND CONCLUSIONS

A pasture at Beltsville, Maryland, was exposed over a period of nearly 6 months in 1940 (May 10 through October) to contamination with sheep feces containing a calculated total of 89,500,000 *Oesophagostomum columbianum* eggs. Two clean lambs grazed upon this pasture for two weeks the following June, after the pasture had been rested overwinter for a period of seven months (November through May), failed to become infected with nodular worms.

The overwinter loss of nodular worm larvae occurred on a closely cropped pasture, during a fairly normal winter and an abnormally dry spring. The weather during the period the pasture was tested for survival of larvae was unusually wet and was considered very favorable for acquisition of infection if living larvae had been present.

It is concluded that in the region of Beltsville, Md., and probably also in areas having similar or more rigorous winters, the perpetuation of nodular worm infection in sheep flocks from year to year is due to the persistence of the adult worms in the intestines of breeding sheep rather than to the survival of the free-living stages of the parasite on pastures.

For the control of nodular worm infection it is recommended that pastures be rested overwinter, and that all breeding stock be given anthelmintic treatment in the winter and/or spring before being put to pasture. This method of control should prove highly effective for the control of nodular worm infection in northern states, and should incidentally help control other intestinal nematodes of sheep.

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NOTES ON THE GENITAL SYSTEM OF THE BIRD FLUKE,
APHARYNGOSTRIGEA CORNU (ZEDER)

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The large intestinal fluke, *Apharyngostrirea cornu* (Zeder), is frequently met with by students of helminthology in routine autopsies of bird hosts. However, due to its greatly thickened, muscular body and the dense masses of vitelline glands, favorable specimens for making whole mounts are infrequently encountered. Therefore, probably most students seldom do more with this trematode than rather accurately guess at its specific identity, label the specimen and add it to their collection. Thus, the writers have undertaken to make a detailed study of the finer structures of the worm. At the outset it may be stated that nothing unusual in the anatomy was met with although many of the details are made clearer and the total topography of the parasite is brought up to date.

We have been rather fortunate in obtaining a large number of these parasites from various species of water birds in Mississippi, Georgia and Tennessee. With an abundance of material at hand we have been able to study the species from whole mounts and a large series of sections.

Apharyngostrirea cornu (Zeder, 1800)

(Figs. 1-3)

The body is sub-cylindrical in cross section, greatly elongated in outline and shows a strong dorsal curvature. The overall measurements* are: length 6 mm; posterior body 4 mm long by 1.32 mm; anterior segment 2.20 mm long by 1.70 mm. The anterior segment is more flattened than the posterior one and is formed as a somewhat flat tube; it overlaps the posterior segment for about 200 μ ventrally.

The body is covered by a relatively thick cuticle, devoid of spines, that dips into all openings. The dorsal curvature, which is marked, is due to a slight contraction of the very well developed muscle layer (Fig. 3, M) which begins anteriorly just behind the oral sucker as a double band and passes into the hindbody. At about the level of the holdfast organ the two bands of muscles become fused into a solid band and this continues into the hindbody. At the level of the reproductive organs the band of muscles becomes dispersed into thin strands and a marginal layer that continue on to the posterior end of the caudal segment.

The oral sucker is subterminal in position and measures about 150 μ in diameter. It leads into a short esophagus that is devoid of a muscular pharynx. The two ceca are slender tubes that diverge around the acetabulum but bend ventrally into the holdfast organ. After emerging from the holdfast organ, the ceca pass into the hind-

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* Only maximum measurements are given in recording the dimensions of the various organs and structures.

body and continue posteriorly somewhat laterally and dorsally to the gonads, terminating in the area adjacent to the bursa.

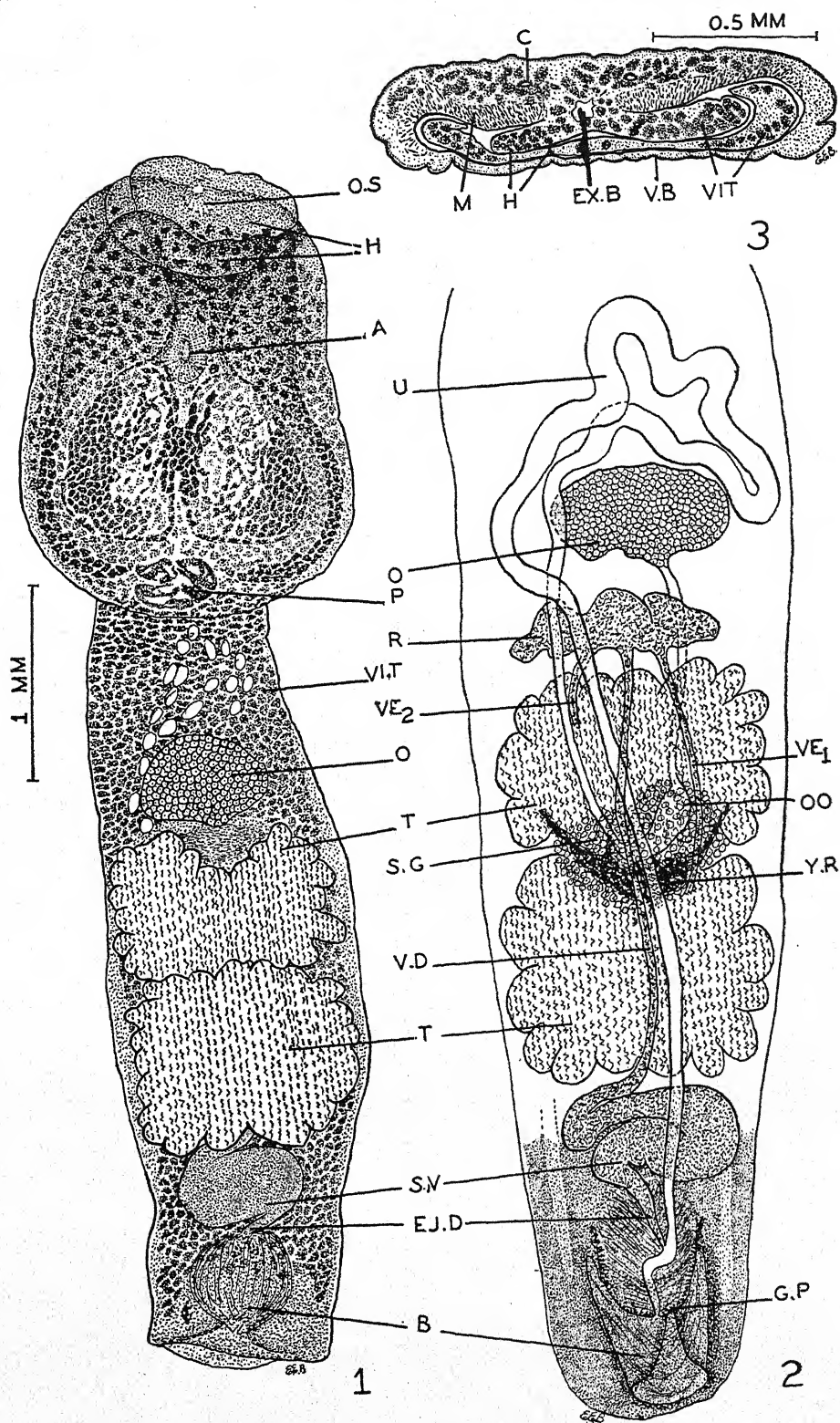
The acetabulum lies approximately $500\ \mu$ behind the caudal boundary of the oral sucker and measures $360\ \mu$ long by $240\ \mu$ wide. In whole mounts the acetabulum is difficult to make out since it is covered by the ventral wall of the anterior segment, the dorsal and ventral lobes of the holdfast organ and is usually withdrawn into a deep groove within the dorsal wall of the cavity of the anterior segment. In sections the acetabulum is seen to be definitely stalked within a groove-like receptacle.

The holdfast organ is enormously developed. It consists of two well developed lobes. The first of these develops from the base-like mass of tissue forming most of the body wall in the posterior limits of the cavity of the anterior segment. From this mass the dorsal lobe originates by an outgrowth (Figs. 1 and 3, H) into the cavity of the anterior segment. Where the lobe becomes detached from the wall two conspicuous folds remain attached to the wall for a short distance and these assist in forming the groove into which the acetabulum can be withdrawn. At about the level of the acetabulum the lobe loses its connection with the body wall and becomes a very much narrowed wedge of tissue. Anterior to the acetabulum the lobe expands again into a thin, flat, tongue-like mass of tissue and this frequently protrudes from the cavity of the anterior segment (Fig. 1, H). The ventral lobe originates from the caudal margin of the base-like mass as a broad, flat fold and follows the ventral wall of the anterior segment to the anterior end. This lobe becomes so folded as to produce a C-like tube, the lateral margins of which incloses the dorsal lobe within its cavity. The ventral lobe frequently protrudes a short distance from the cavity of the anterior segment (Figs. 1 and 3, H). Both lobes of the holdfast organ contain follicles of the vitellaria. The dorsal lobe is free of vitellaria anterior to the acetabulum but the ventral lobe is crowded with these glands to its anterior boundary.

At the base of the holdfast organ is located a large proteolytic gland. The gland consists of about six large, compact lobes which appear as clusters of deeply staining masses of cells surrounding a central vascular-like area. The entire mass may measure as much as $400\ \mu$ in diameter.

The posterior segment of the body is sub-cylindrical and strongly curved dorsally. It tapers toward both ends, being widest in the region of the testes, slightly caudal to the middle of the body. Most of the reproductive system is located within the posterior segment.

The female reproductive system. The transversely ovoid ovary, $460\ \mu$ long by $640\ \mu$ wide, lies across the midline in the hindbody about $650\ \mu$ posterior to the proteolytic gland. It gives rise to a long, slender oviduct which passes posteriorly along the midline, dorsally to the anterior testis to the intertesticular area. Here the oviduct becomes dilated into the oötype (Fig. 2, OO) and receives the ducts from the shell gland and yolk reservoir. The shell gland (Fig. 2, S.G) is a conspicuous mass of cells surrounding the oötype and proximal end of the uterus, and fills in most of the space between the testes. Beyond the shell gland and oötype the uterus bends ventrally and anteriorly to ascend over the ventral face of the anterior testis. In the region of the ovary the uterus becomes convoluted as it passes forward around the ovary and up to the anterior end of the posterior body segment, swinging across the body to the opposite side in ascending. From its anterior limit the uterus makes several convolutions back across the body to the same side on which it ascended,

*Apharyngostrigea cornu* (Zeder, 1800)

then descends through an almost straight course, along the mid-ventral aspect to the genital pore. In the region of the pore the uterus makes a conspicuous, right angle bend, becomes dilated somewhat and then opens into the bursa. The ova are fairly numerous within the uterus. They measure $118\ \mu$ long by $70\ \mu$ wide. The vitellaria are follicular and distributed throughout most of the body. In the forebody the vitelline glands occupy most of the area of holdfast organ and the dorsal wall of the anterior segment, being absent from the ventral wall of that segment. They reach from the level of the oral sucker to the caudal boundary of the forebody. In the hindbody the glands form a dense, ventral mass stretching across the body from its beginning to the ovary. At the ovary the vitelline glands become restricted into two narrow bands that extend down the hindbody, ventrally and laterally through the region of the testes and then fuse into a solid band in the ventral and lateral aspects posterior to the testes. They terminate just posterior to the ends of the ceca, in the area of the bursa (Figs. 1 and 3, VIT). In the area of the anterior testis, two yolk ducts converge toward the midline at the intertesticular area to form a yolk reservoir (Fig. 2, Y.R). The common yolk duct is short. It passes anteriorly from the reservoir to enter the uterus near the point where the latter emerges from the oötype.

The male reproductive system. The testes are large, conspicuous bodies lying in the hindbody, caudal to the ovary. They are arranged in tandem. In sections, both testes appear as bi-concave structures with their margins meeting dorsally and ventrally. In outline both testes are deeply indented to irregular. They measure $720\ \mu$ long by $1.16\ \text{mm}$ wide for the anterior and $880\ \mu$ long by $1.20\ \text{mm}$ wide for the posterior testis. The anterior testis is separated from the ovary by the seminal reservoir. Each testis gives rise to a vas efferens from its antero-ventral surface, one on either side of the midline (Fig. 2, VE_1 , VE_2) and these pass forward, ventrally to the testicular bodies. The two vasa efferentia unite in the area between the ovary and anterior testis and form a greatly expanded seminal reservoir. The reservoir fills the space between the testis and the ovary and may extend across the entire width of body in that area. From the seminal reservoir the vas deferens (Fig.

FIG. 1. Ventral view of entire specimen (later sectioned, from which most of the sections studied were taken), showing location and relative proportions of all internal structures. Drawn with aid of camera lucida.

FIG. 2. Reconstruction of organs and organ systems in the posterior segment of the body.

FIG. 3. Cross section (drawn with aid of camera lucida) through anterior segment of body just below the level of the acetabulum, showing the wall of the forebody and the two lobes of the holdfast organ. Note the distribution of the vitelline glands in the section.

ABBREVIATIONS

A—acetabulum	P—proteolytic gland
B—bursa copulatrix	R—seminal reservoir
C—cecum	S.G—shell gland
EJ.D—ejaculatory duct	S.V—seminal vesicle
EX.B—excretory vesicle	T—testis
G.P—genital papilla	U—uterus
H—holdfast organ	V.B—ventral body wall
M—muscle	V.D—vas deferens
O—ovary	VE_1 —posterior vas efferens
OO—oötype	VE_2 —anterior vas efferens
OS—esophagus	VIT—vitellaria
	Y.R—yolk reservoir

2, V.D) descends along the midline and in close proximity to the descending uterus to the area just caudal to the posterior testis. At this point the vas deferens becomes dilated and thrown into a series of folds, loops and turns, becoming more and more expanded. Then suddenly the tube becomes constricted to form a short ejaculatory duct (Figs. 1 and 2, E.J.D). The expanded portion of the vas deferens, the seminal vesicle, is filled to capacity with spermatozoa. The ejaculatory duct merges with the uterus at the angle where the latter structure makes its sharp bend before passing to the bursa.

In most whole mounts the bursa appears to be terminal although in sections it is seen to be slightly dorsal in position. The bursa is well developed and is eversible. A wide slit-like opening communicates the bursa to the outside. Internally the bursa is lined with cuticle and is surrounded by well developed muscles. A well developed genital papilla may be protruded into the bursa or withdrawn into the body. When protruded, and frequently the papilla can be seen protruding through the bursal opening to the outside, the papilla is seen to give vent to the reproductive tract, with the so called "hermaphroditic" canal (that part of the reproductive canal from the union of the ejaculatory canal with the uterus) opening at the summit. No prostatic gland cells were observed.

The above description of *Apharyngostrigea cornu* is based on a study of material taken from the little blue heron, *Florida caerulea caerulea*, from Mississippi. Other hosts from which specimens have been taken include Ward's heron, *Ardea herodias wardi*, American egret, *Casmerodius albus egretta*, eastern green heron, *Butorides virescens virescens*, and black-crowned night heron, *Nycticorax nycticorax hoactli*.

SUMMARY

The bird fluke *Apharyngostrigea cornu* (Zeder, 1800) is reported from a number of water birds, little blue heron, eastern green heron, Ward's heron, black-crowned night heron and American egret. The finer details of its internal anatomy are described and illustrated.

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ON THE VIABILITY OF VARIOUS SPECIES OF *TRYPANOSOMA* AND *LEISHMANIA* CULTURES

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Most species of trypanosomes grow readily on original N. N. medium (3) or on its various modifications such as N. N. N. and regular blood agar slants which contain from 10% to 50% of defibrinated blood (1, 2, 4).

After the initial culture of a given species of trypanosome is obtained from vivo into vitro, the subsequent subcultures when made on N. N. medium every second or fourth week usually give luxuriant growths of trypanosomes not only in the water of condensation but also on the slant portions of the blood agar tubes (1, 2, 3, 4, 5, 6, 9).

The present study summarizes the results of the microscopic examination the stock subcultures for the viability (motility) of *Trypanosoma cruzi* and other flagellates on culture medium in vitro stored at 18°–31° C from two months to as long as six years.

METHOD AND MATERIAL

Cultivation and transplantation of various strains of Trypanosoma and other flagellates in vitro.—From 1928 to 1942 fifteen species of trypanosomes representing over 250 strains were cultured by the writer on N. N. medium (4, 5, 6, 7, 8). Of these strains over 100 were *Trypanosoma cruzi*, some of which were isolated as early as 1932 (4). In addition to the above, four species of flagellates such as *Leishmania* and *Herpetomonas* were received from other investigators.² The stock cultures of the above species and strains were maintained in vitro, by monthly subculture for many generations, the minimum period for each strain in vitro being five months and the maximum over ten years.

Storage of Trypanosoma and Leishmania cultures.—The stock cultures of the flagellates in N. N. tubes inoculated during 1936 to August, 1941, were kept at the National Institute of Health, U. S. Public Health Service, Washington, D. C., in a room with a temperature fluctuation of from 18°–30° C. In October, 1941, these cultures were brought to Galveston, Texas, and during the winter and spring were kept at room temperatures of from 20°–27° C and during July and August of from 28°–31° C. The most recent transfer of stock cultures as a rule was kept at a constant temperature of 25° C for three months before being stored at room temperature.

Examination for viability of trypanosomes.—In order to ascertain the viability of trypanosomes at various periods of time, a loop of material was removed from the culture medium (usually from the water of condensation) and examined micro-

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¹ Part of this work was performed at the National Institute of Health, U. S. Public Health Service, Washington, D. C., during 1936–41.

² Strains of *Leishmania donovani* or *L. tropica* or both were received from Professor F. G. Novy, University of Michigan; Doctor D. Berberian, American University of Beirut; Doctor B. M. Das Gupta of Calcutta; a strain of *Herpetomonas* was received from Doctor R. W. Glaser of Princeton, N. J., and a strain of *Trypanosoma vespertilionis* from Doctor E. Dias, Brazil, South America.

scopically (objective 45 \times , ocular 10 \times) under cover-glass preparation. (To facilitate making the large number of examinations required, cover glasses 22 \times 22 mm were each cut into four pieces with a diamond pencil, thus making possible eight examinations on one slide.) A majority of the experimental tubes was examined within three days; the stock cultures, on the other hand, were examined at various intervals. Usually from one to three minutes were devoted to microscopic search of each culture tube. With few exceptions the cultures which were negative by microscopic examination were not subjected to the cultural test to ascertain whether or not a scanty number of viable trypanosomes may have been present.

DATA

The experimental data on hand show that *Trypanosoma cruzi* invariably remain actively motile on N. N. medium during the first two and even three months. Out of 520 tubes examined, 124 cultures kept in vitro for approximately six years gave 13 positive results; 290 kept from seven months to five years yielded 11 positives; and the remaining 106 kept from January to June, 1942, gave 68 positives, making a total of 92 tubes containing actively motile trypanosomes.

It is noteworthy that the contents of the tubes kept at room temperature for as long as six years were still in good condition, and the majority of the tubes even then contained 0.5 cc or more of the water of condensation. Although the formula for preparing blood agar slants (N. N. medium) was always about the same, the addition of rabbit's blood to the medium varied from 20% to 50%. This variation, together with the different lots of rabbit blood, the infusion broth, and the pH, may possibly have caused the higher percentage of positive findings as obtained from certain lots of culture tubes.

The record of the positive findings in the present study is based chiefly on actual demonstration by microscopic examination of trypanosomes in the culture tubes. In some instances when trypanosomes were not seen, positive cultures were obtained from such tubes by inoculating a few drops of the water of condensation from the N. N. tubes into new culture medium. However, this procedure was not utilized extensively in the present study. It appears that the percentage of positive findings possibly would have been higher if subcultures had been made from each microscopically negative culture tube or if more time had been devoted to microscopic search. Whenever the trypanosomes were demonstrated microscopically, the subcultures always gave positive results.

It is observed that *Trypanosoma cruzi* remain viable for longer periods on N. N. medium than do other species of trypanosomes. Based on over one hundred cultural observations, the viability of *T. avium* on blood agar slants in vitro was found to be usually one but rarely more than three months; similarly, *T. rotatorium*, about three to four months, provided the cultures were kept at a room temperature of not higher than 25° C. A limited number of subcultures of *T. americanum*, *T. duttoni*, *T. lewisi*, *T. melophagium*, *Leishmania donovani*, and *L. tropica* was found to yield negative results at the end of four months; however, in many instances subcultures were obtained at the end of two and occasionally during the third month. In one instance *Herpetomonas musca domestica* were found viable and actively motile on N. N. medium after 2066 days (about six years).

As the cultures grew older, some of the flagellates gradually became pear-shaped and rounded in form. The rosettes assumed a granular appearance in small and large aggregations, which eventually led to disintegration and death.

Fifteen species of trypanosomes and four other species of flagellates (representing over 250 strains) kept on blood agar slants (N. N. medium) were found to be actively motile when one month old. Subcultures from such tubes nearly always yielded 100% positive results.

SUMMARY

Out of 124 subcultures of *Trypanosoma cruzi* kept in vitro for approximately six years without further subculturing, 13 tubes were found still to contain actively motile trypanosomes; 291 culture tubes kept from seven months to five years gave 11 positive results, and the remaining 110 cultures kept about six months presented 68 positive findings.

The viability of *T. avium* on blood agar slants in vitro was found to be usually one but rarely more than three months, and of *T. rotatorium* about four months when the cultures were kept at room temperature not higher than 25° C. A limited number of subcultures of *T. americanum*, *T. duttoni*, *T. lewisi*, *T. melophagium*, *Leishmania donovani* and *L. tropica* was found to yield negative results at the end of four months; however, in many instances subcultures were obtained at the end of two and occasionally during the third months. *Herpetomonas musca domestica* in one instance were found to be viable on blood agar slants after a period of six years.

Fifteen species of trypanosomes and four other species of flagellates (representing over 250 strains) kept on blood agar slants (N. N. medium) were found to be actively motile when one month old. Subcultures from such tubes nearly always yielded 100% positive results.

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ANAEROBIOSIS AND CHOLESTEROL AS GROWTH REQUIREMENTS OF *ENDAMOEBA HISTOLYTICA*

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Since the introduction of methods for the in vitro cultivation of *Endamoeba histolytica*, the value of this procedure has been adequately demonstrated. It has been generally felt, however, that pure cultures, free of the accompanying bacteria, could be applied even more widely to problems of practical interest. The primary obstacle to the purification of *E. histolytica* cultures, that of separating the amoebae in a viable state from all living bacteria, has been overcome by several methods (Cleveland and Sanders, 1930; Rees, 1939; Meleney, Frye, Leathers and Snyder, 1940). In no case, however, was it possible to establish transferable cultures in sterile media with the bacteria-free preparations obtained. Since prompt development occurred upon the addition of bacteria, it is apparent that the association of *E. histolytica* with bacteria is functional rather than merely coincidental.

The nature of this association might be either simple or complex, and dependent upon one or more factors. The preliminary experiments of Cleveland and Sanders (1930) and our own (Meleney and others, 1940) have shown that a wide variety of sterile media, including culture filtrates and heat-killed bacteria, would not replace living bacteria as a requirement for either excystation or growth. It has also been found (Chinn, Jacobs, Reardon and Rees, 1942) that in certain cases, mixed cultures of two or three bacterial species induce better growth of *E. histolytica* than do pure cultures of the same species. These observations do not favor a simple explanation for the dependency of the amoebae upon bacteria, and the data to be presented in this report indicate that several factors are actually involved. If the number of significant factors should prove to be large, the possibility of providing a suitable combination by any empirical method obviously becomes remote. In order to simplify this situation, we have attempted to divide the study into several phases, each of which could be investigated separately. In this way we have already shown (Snyder and Meleney, 1941) that the requirements for excystation are suitable anaerobic conditions, ordinarily provided by the metabolic activity of bacteria. These conditions alone are not sufficient for continued multiplication of the excysted trophozoites, and various nutrient media did not supply the deficient factors.

In pursuance of this procedure of investigation, we have undertaken the analysis of the growth requirements of *E. histolytica* through a study of cultures containing bacteria. The object of the present report is to describe the progress made in this study to date.

All exploratory experiments were carried out with cultures of the Dobell (1931) monkey strain of *E. histolytica* (Strain NRS), but significant findings have been confirmed with six other strains. All culture media were employed in one milliliter volumes in culture tubes of 16 millimeter diameter, thus providing a large surface-

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volume ratio in order to emphasize any contrast between cultures incubated under aerobic and anaerobic conditions. Each tube of medium was inoculated with one drop of thoroughly mixed culture, thus providing large and relatively uniform inocula. Since essential growth factors might also be transferred under these conditions, at least five consecutive transfers in any medium were considered necessary to establish its suitability for the cultivation of *E. histolytica*. Cultures were examined directly through the wall of the culture tube placed on the stage of an horizontally inclined microscope, but the validity of this method was confirmed by slide examinations. Starch was provided as approximately two milligrams of completely dispersed grains of rice starch purified by digestion of the rice flour proteins with trypsin and repeated washing with water and fat solvents.

Although it appeared probable that the final interpretation of results obtained with mixed cultures of *E. histolytica* and bacteria might be open to question, such cultures might supply suggestive evidence bearing on several problems of immediate interest. On the one hand, since the media previously used for the cultivation of this amoeba are relatively complex, it was considered desirable to make available a simpler medium prepared with constituents of nearly constant composition, and if possible to determine the growth-essential factors provided by the medium. Following the observation of Reardon and Rees (1939) that coagulated whole egg supplies all of the organic growth requirements of *E. histolytica* cultures containing bacteria, we were able to replace this by an entirely liquid medium prepared by infusing coagulated whole egg in 0.8 per cent sodium chloride. This medium gave approximately as abundant growth as comparable media containing coagulated egg slants. Growth was heavier and more persistent if starch was added, but cultures could be maintained indefinitely without starch if transferred daily. This egg infusion medium served as a starting point for the analysis of the growth requirements of *E. histolytica* in the presence of bacteria, and as a control for comparison with the results obtained.

On the other hand, the probable significance of anaerobic conditions for the growth of *E. histolytica* had been suggested by the necessity of anaerobic conditions for excystation and by the fact that an essentially anaerobic environment predominates in cultures of oxygen-consuming bacteria (Rahn and Richardson, 1941, 1942) and in the usual habitat of the amoeba (the lower intestine). However, it is obvious that in aerobic cultures with bacteria, the amoeba are exposed to an environment which is neither strictly aerobic nor strictly anaerobic, and thus is difficult to evaluate. We chose to simplify this situation by imposing a strictly anaerobic environment upon the whole culture through absorption of the oxygen with alkaline pyrogallate sponges in the culture tubes. In egg infusion, growth of *E. histolytica* occurred for one or two transfers under these conditions, but never for three consecutive transfers. The failure of these cultures could not be ascribed to a direct effect of the lack of oxygen upon the amoebae, since growth in the first anaerobic culture was quite abundant. Furthermore, it was noted that the turbidity developing from the bacteria transferred with the amoebae was distinctly lessened after a few transfers under anaerobic conditions. This suggested the importance of large numbers of bacteria for the growth of *E. histolytica*, a requirement which had previously been demonstrated for *Dientamoeba fragilis* by Brug (1936). Consequently, anaerobic cultivation was again attempted, but each transfer was made, not into sterile medium, but into bacterial cultures which had been previously inoculated and incubated

aerobically for 24 hours. By this method, anaerobic cultures of *E. histolytica* could be maintained for an indefinite number of transfers. The most satisfactory bacterial culture found for this purpose was a mixed culture obtained by destroying the amoebae present in aerobic cultures of *E. histolytica*. This bacterial culture was maintained separately by daily transfers in egg infusion. Pure cultures of several bacterial species gave either negative or poor results. Heat-killed cultures were inactive.

After a few transfers under these conditions, anaerobic cultures of *E. histolytica* showed several characteristics which remained constant throughout the period of study. Multiplication was detectable after about 24 hours, and in two days the population reached a maximum roughly comparable to that found under aerobic conditions. Starch was required, probably as a source of carbohydrate, in these and in all other anaerobic cultures, and could not be omitted for a single transfer. On the other hand, it was possible to make occasional transfers to sterile medium rather than to a bacterial culture, but this could not be repeated for several consecutive transfers. Anaerobic cultures inoculated into sterile medium and incubated aerobically also failed to develop, unless a heavy inoculum of bacteria was added at the time of inoculation. In the latter case, there resulted a typical aerobic culture which could be maintained indefinitely under aerobic conditions by transfers into sterile media, indicating that anaerobic cultivation had at least induced no permanent change in the amoebae. In addition, anaerobic cultures showed a marked susceptibility to exposure to air. Slide examinations demonstrated that death and degeneration of practically all amoebae present in luxuriant cultures had occurred within one to three hours after opening the tubes. It was sometimes possible to obtain subcultures after this period, due perhaps to the presence of a few cysts. These observations indicate that bacteria in large numbers are required not only for the growth of *E. histolytica*, but even for the survival of trophozoites when air has access to the cultures.

TABLE 1.—Analysis of minimum cultural requirements of *Endamoeba histolytica*

	"Aerobic" conditions	Anaerobic conditions			
		1	2	3	4
Media (1.0 ml)	Sterile egg infusion	Whole, living, aerobic, mixed- bacteria culture in egg infusion	Living, washed, mixed- bacteria	Fresh, sterile cysteine	Fresh, sterile cysteine
			Sterile mixed- bacteria culture filtrate	Sterile peptone- Ringer's solution	Sterile peptone- Ringer's solution
				Sterile egg infusion	Sterile cholesterol
		Sterile rice starch			
Inoculum (1 drop)	<i>Endamoeba histolytica</i> and bacteria				

The analysis of the growth requirements of *E. histolytica* under anaerobic conditions proceeded as indicated by Media 1 to 4 of Table 1. It was found that the activity of the bacterial culture (Medium 1) resided neither in the bacteria alone, nor in the fluid portion alone, since neither washed, living bacteria nor culture filtrate added separately to sterile egg infusion provided a suitable substratum for anaerobic cultivation. However, the activity of the culture had not been destroyed by washing or by filtration, since reconstitution of the culture (Medium 2) permitted anaerobic growth. This is a clear indication that more than one growth requirement was supplied through bacterial activity. Therefore, the two fractions were studied separately.

Washed, living bacteria could not be replaced by heat-killed bacteria, but it was found possible to substitute sterile reducing agents for this fraction (Media 3 and 4). Of the reducing agents tested to date, cysteine permitted quite as abundant growth as did the bacterial fraction, "reduced iron" gave distinctly poorer results, and thioglycollate, sulfide, sulfite, formaldehyde-sodium sulfoxylate and zinc metal were found inactive. To a certain extent, therefore, the requirements for growth parallel those for excystation (Snyder and Meleney, 1941), since in both cases elimination of oxygen and the presence of reducing agents appear to be essential. However, the requirements for growth are apparently more specific, in that thioglycollate, which had induced excystation quite as readily as cysteine, did not permit growth in the present situation. It is probable that the sole function of the accessory bacteria added as the bacterial fraction is that of reducing activity due to bacterial enzymes, but is not known whether this activity is concerned only with hastening the removal of traces of oxygen dissolved in the medium, or whether it also includes other essential oxidation-reduction reactions.

The filtrate fraction, on the other hand, apparently supplied at least two growth-essential factors, since it was replaceable only by a combination of substances. These factors could be provided by a mixture of sterile Difco Proteose-peptone in Ringer's solution and sterile egg infusion (Medium 3).

Sterile egg infusion was replaced by cholesterol (Medium 4), but the significance of this compound requires further qualification. Substitution alone is not sufficient to prove that cholesterol and the active factor provided by egg infusion are identical. However, comparison of the lowest concentration of chemically pure cholesterol permitting growth (between 1:1 million and 1:10 million) with the growth-limiting dilution of an ether-extract of egg yolk (between 1:50 thousand and 1:150 thousand of the original yolk) and the known cholesterol content of egg yolk (1:100 to 1:300) suggests that cholesterol is the active substance in egg, since these values approximate within reasonable limits. The few related substances which were tested (bile salts and non-irradiated ergosterol) were found inactive. The observations of Dopter and Deschiens (1938) on the effect of cholesterol and bile salts on the growth of *E. histolytica* were made in a medium which must have completely satisfied any requirement for cholesterol, and thus are not especially pertinent to the present study. This requirement is not entirely specific, however, since we were able to replace cholesterol by an ether-extract of rice flour. This is in agreement with the results of Cailleau (1937) who found cholesterol and certain plant sterols interchangeable as growth requirements for several species of flagellates. The sterol content of rice flour also may explain a few reports of the cultivation of

E. histolytica in media lacking egg yolk, blood serum or other obvious source of cholesterol (Cleveland and Collier, 1930; St. John, 1932; Tsuchiya, 1932, 1934). Our own starch preparation was treated to remove sterols, and our experiments were checked by controls to which cholesterol was not added.

Ringer's solution was used routinely, but the individual salts which it contains have not been tested to determine whether or not they are all essential. Peptone could not be omitted, but liver extract or meat extract were equally satisfactory. The significance of this fraction is not entirely clear. Superficially, it might be presumed to act merely as a source of suitable organic carbon and nitrogen compounds. If so, egg infusion must be considered deficient in these compounds until acted upon by living bacteria under aerobic conditions (Media 1 or 2), since the addition of washed bacteria alone to sterile egg infusion without a period of aerobic incubation did not provide a suitable substratum for anaerobic cultivation.

Conversely, the cysteine-peptone-cholesterol medium (Medium 4) was not found suitable for aerobic cultivation of *E. histolytica*, regardless of the type of culture employed as the inoculum, although the turbidity due to bacterial growth was not appreciably less than in aerobic egg infusion cultures. Continued aerobic cultivation was accomplished, however, by simultaneous inoculation with washed bacteria *at each transfer*. It seems probable, therefore, that the failure of the peptone-cholesterol medium in the presence of air is related in some way to the reducing activity of the culture, since this was identified as the function of supplementary washed bacteria under anaerobic conditions.

In addition to the requirements already indicated, anaerobic *E. histolytica* cultures contain a residual bacterial flora transferred with the inoculum, which is also essential for growth. The need for these bacteria is shown by our failure to obtain pure *E. histolytica* cultures in any of our simplified media by inoculation with bacteria-free cysts. The essential nature of these bacteria introduced the possibility that the growth requirements which have been described are related to these bacteria rather than directly to *E. histolytica*. An unqualified decision cannot be made on this point as yet. However, the rapidity with which death occurs in exposed anaerobic cultures is most readily explained as a relatively direct action of oxygen upon the amoebae, and our results as a whole suggest a degree of obligatory anaerobiosis on the part of *E. histolytica* comparable to that of the most sensitive bacterial anaerobes. In regard to cholesterol, it appears quite unlikely that this substance is a growth requirement of essential bacteria. Of the numerous species of bacteria found by Chinn, Jacobs, Reardon and Rees (1942) to be capable of supporting the growth of *E. histolytica* when introduced as pure cultures, many are known not to require cholesterol or related substances, at least in concentrations significant to the present study. We have obtained anaerobic cultures containing only *E. histolytica* and a single species of obligatory anaerobic bacteria isolated from the anaerobic cultures previously described. Cholesterol had no effect on this bacterium in pure culture, and is therefore presumed to be required by the amoebae, either as such, or after chemical alteration by the bacteria. This partially purified culture also required the addition of blood serum to the medium in order to obtain any growth of *E. histolytica*. Since pure cultures of the bacterium grew luxuriantly in the absence of serum, this requirement also may probably be referred directly to the amoebae. The serum factor appears to be heat-labile, but has not yet been studied further. The

presence of serum has so far prevented our testing these cultures directly for their reaction to cholesterol, but there is nothing to suggest that this requirement might have disappeared under these conditions. At least one additional growth factor, perhaps of a supplementary nature, is suggested by the fact that *E. histolytica* cultures containing only this single bacterial species produced only about half the number of amoebae found in anaerobic cultures with mixed bacterial species, and were characterized by a prolonged lag period of about six days. Some improvement was observed in the presence of liver extract, casein or additional peptone, but these results were irregular.

The growth requirements of *E. histolytica*, as far as they are known or have been suggested in the preceding discussion, are outlined tentatively in Table 2. Although

TABLE 2.—Probable growth requirements of *Endamoeba histolytica*

	Factor	Former source	Present source
1	Water		
2	Inorganic salts	Ringer's solution, egg, etc.	Ringer's solution, peptone
3	Amino acids (?)	Egg, serum, etc.	Peptone
4	Carbohydrate (?)	Rice flour	Rice starch
5	Cholesterol	Egg, serum, rice flour	Cholesterol
6	Elimination of oxygen	Bacteria	Alkaline pyrogallate
7	Other reducing activity (?)	Bacteria	Cysteine
8	Heat-labile factor	Bacteria	Serum
9	Other essential factors (?)	Bacteria	Anaerobic bacterium
10	Supplementary factors (?)	Bacteria	Liver extract, casein, etc. (?)

they are listed as the complete or partial requirements of an hypothetical pure culture of the amoebae, it is obviously impossible to test these suggestions in an unequivocal manner until the residual living bacteria are replaced by the remaining unknown factors which they supply. Our results may, therefore, be found capable of other interpretations, but it appears desirable at the present time to regard most of the factors listed as distinct growth requirements of *E. histolytica*, and to base further study on this hypothesis.

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SURVIVAL ON GRASS PLOTS OF EGGS AND PREINFECTIONAL LARVAE OF THE COMMON SHEEP STOMACH WORM, *HAEMONCHUS CONTORTUS*

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Attempts to control the common stomach worm, *Haemonchus contortus*, have been directed chiefly toward the elimination of the parasites by the use of medicinal agents. Another method of control, namely, pasture rotation, has been advocated, but in order for this method to be successful, it must be based on a thorough knowledge of the free-living stages of the parasites and on the conditions which influence the embryonation of eggs and the development and survival of the larvae. Of the various preparasitic stages in the development of the parasite, the greatest attention has been paid to the third stage or infective larvae because they are capable of infecting sheep. However, since the infective larvae can develop only from preinfective larvae, the factors that influence the development and survival of these stages are likewise important. It is the purpose of this paper to present data on the influence of temperature, sunlight, and moisture on the survival of the preparasitic stages of *H. contortus*.

HISTORICAL REVIEW

Veglia (1915) studied the effect of high and low temperatures, drying and sunlight on the preinfective stages of *Haemonchus contortus* and found that stomach worm eggs kept in the dark at 26° C under favorable moisture conditions reached the tadpole stage in 6 hours; 25 per cent of the eggs hatched in 14 hours, 50 per cent in 17 hours and the majority of the remainder in 48 hours. This temperature might be considered as optimum for embryonic development. According to Veglia, deviation from this optimum caused either a slowing of embryonic development or death. Some eggs kept at 50° C survived for two hours but all were dead in four hours. Eggs in feces kept at 40° C invariably failed to develop as did 2 of 6 cultures kept at 37° C. At 15° to 18° C development was normal but slowed; at 8° C larvae in fecal cultures reached the second stage. No change took place in eggs kept at 4° C

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and all eggs in feces placed in a layer between blocks of ice were dead in 24 hours. Veglia also found that when *Haemonchus* eggs in feces were placed in sunlight during summer they became devitalized within two days and in some lots in less than one day. His attempts to culture fecal pellets containing numerous eggs always failed when the fecal material was mixed with sufficient water to reduce the pellets to the consistency of pulp. Immature larvae and eggs, except when in the vermiform embryo stage, did not resist drying. Some eggs in the vermiform embryo stage apparently were able to survive in dried feces for as long as 20 days. Mönnig (1930), on the other hand, found that all preinfective stages of *H. contortus*, including the vermiform embryo, were killed by drying.

MATERIALS AND METHODS

In the experiments reported in this paper, 4-foot square grass plots, each divided into 2-foot square subplots, were sowed with a mixture of pasture grasses and white clover. After the herbage had reached a height of four inches, feces containing eggs and larvae were placed on the subplots. When the period of experiments was completed the grass was cut and washed, and the washings were examined for infective larvae. Larvae in the soil were recovered by means of the Baermann apparatus, the first 25 cc of water drawn from the Baermann funnel after 48 hours being examined for larvae.

EXPERIMENTAL DATA

Experiment 1: Survival of Haemonchus contortus Eggs in December, 1936, February, 1937, and March, 1937

Grass sowed in the fall of 1936 was trimmed at weekly intervals until the onset of cold weather. Fresh feces containing the eggs were placed in the center of each subplot of two plots in December, 1936, in the subplots of a third in February, 1937, and of a fourth in March, 1937. Cultures made at the same time and from the same lots of feces used on these plots were incubated at room temperature and examined two weeks after preparation. The data of this experiment are shown in Table 1.

TABLE 1.—Effect of winter weather on the survival on grass plots of the eggs of *Haemonchus contortus* in the feces

Date of preparation of subplots	Amount of feces placed in each subplot	Eggs per gram of feces	Minimum temperature in °F on 4 days after exposure				Larvae recovered		Larvae recovered from 100 grams control cultures
							4-28-37	5-6-37	
	grams	number	1st	2nd	3rd	4th	number	number	number
12-14-36	200	2,200	28	30	31	40	0	0	186,000 to 194,000
12-21-36	200	1,600	32	29	22	26	0	0	54,510
2-10-37	266	19,200	27	20	31	38	0	0	446,520 to 511,090
3- 5-37	533	11,200	42	37	34	38	0	0	926,520 to 1,208,850

These data show that apparently the embryos in the eggs of *H. contortus* were destroyed by exposure to cold weather. As no larvae were recovered from the 2 subplot examinations, the remaining subplots were not sampled.

Experiment 2: The Protection against Sunlight, Heat, and Drying Afforded the Eggs and Larvae of H. contortus by Grass

On August 5, 1937, 50 grams of sheep feces containing 13,800 eggs of *H. contortus* per gram were placed on each of two subplots. Two weeks later 376 larvae

were recovered from the grass and 988 larvae from a one-inch layer of soil from one of the subplots. Just after removing the grass and soil from this subplot, 100 grams of a 4-day old fecal culture were added to each subplot. Two 100-gram samples from the same lot of feces used in preparing the culture yielded by the Baermann technique an average of 27,265 second and third stage larvae. The feces on the two subplots were exposed in the afternoon. A light sprinkle of rain fell 4 hours later, and during 6 of the succeeding 7 days there was a total rainfall of 5.39 inches. The plot was exposed to sunshine only 47 per cent of the possible maximum time, but in spite of this relatively small amount of sunlight the feces and the soil to a depth of one inch when examined at the end of one week's exposure yielded only 21 larvae. The control plot yielded 6,070 larvae, of which about 5,000 probably came from the second inoculation. This experiment shows that second and third stage larvae cannot survive in numbers on bare ground in summer even in wet cloudy weather.

Experiment 3: The Effect of Moisture and Consistency of Feces on the Survival of Preparasitic Eggs and Larvae

In July, 1936, 100 grams of feces containing eggs of *H. contortus* were placed on the 20 subplots of 5 grass plots. Feces were applied in a different manner to each plot as shown in Fig. 1. Twelve 100-gram control cultures kept in the laboratory until the plots were examined each yielded from 434,000 to 1,031,000 larvae.

Three of the subplots (a, b, and c) of each plot received water applied with a sprinkling can. Subplots "a" of each plot received one quart of water daily in addition to whatever rain fell, subplots "b" one-third of a quart daily, while subplots "c" each received one-third of a quart of water at weekly intervals. The water added to these subplots during the 18 days the experiment lasted was approximately equivalent to 1.8, 0.54 and 0.07 inches of rain, respectively. Subplots "d" received no water other than that due to rainfall. Rain totalling 0.47 inch fell on 4 of the 5 days previous to the test period, while on 9 days of the period of experiment the rainfall amounted to a total of 3.5 inches. The temperature ranged from 60° to 90° F, with a daily mean of 69° to 86° F. The sun was shining an average of 65 per cent of the possible maximum time.

The number of larvae washed from the grass cut from the 20 subplots is shown in Table 2.

TABLE 2.—*Effect of condition of feces, of manner of their distribution on soil surface, and of moisture on survival of eggs and larvae*

Feces applied to subplots as*	Distribution of feces on soil surface	Amount of water added to subplots after feces were applied				Total larvae recovered
		1 qt. daily	0.33 qt. daily	0.33 qt. weekly	None (control)	
		Larvae recovered from grass cut from subplots 18 days after the feces had been applied				
		<i>number</i>	<i>number</i>	<i>number</i>	<i>number</i>	<i>number</i>
Whole pellets	Scattered over area	20,856	246	706	59	21,867
	In a pile in center of area	7,212	714	1,150	259	9,335
Crushed pellets	Scattered over area	5,018	1,648	1,512	345	8,523
	In a pile in center of area	14,701	12,075	2,228	420	29,424
Watery paste	In center of area	15,558	6,464	7,050	1,565	30,637
Total		63,345	21,147	12,646	2,648	

* The amount of feces in all cases was 100 grams.

As the larvae of *H. contortus* under favorable conditions reach infectivity in 4 or 5 days, it may be noted that this experiment tested not only the survival of pre-infective larvae and eggs but the survival of infective larvae as well. The difference between the number of larvae recovered from the grass and the number recovered from the control cultures represents the number of larvae left on the grass stubble, in the debris and feces on the soil surface, and in the soil, as well as the eggs and

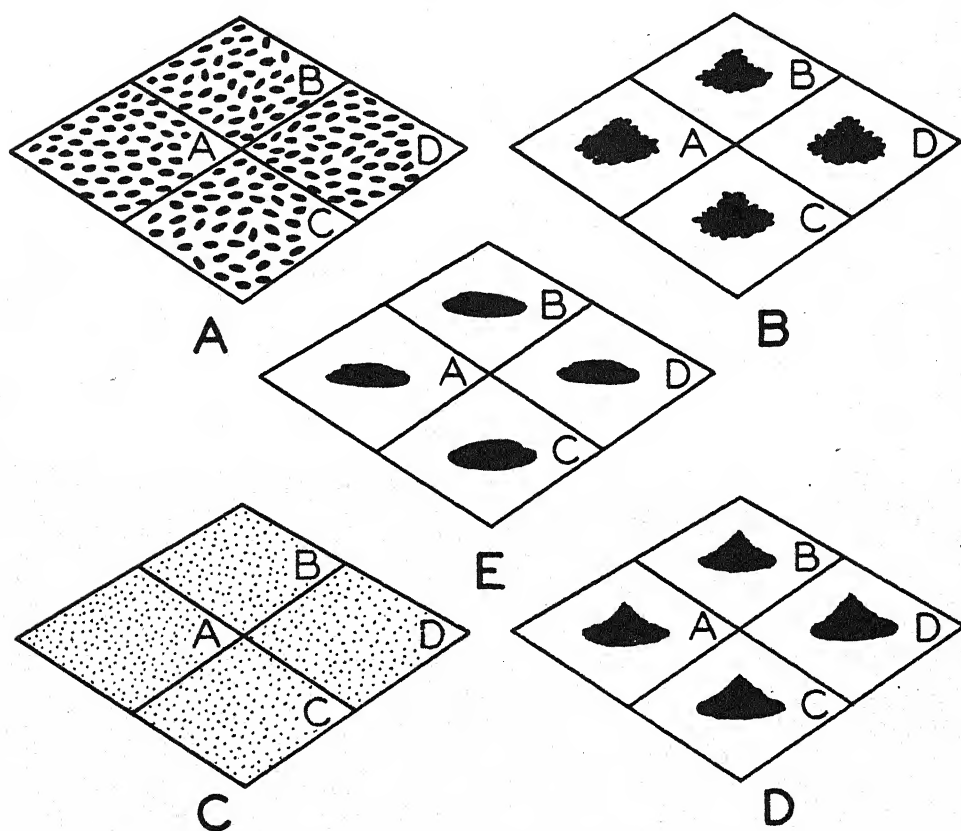


FIG. 1. Diagram showing distribution of sheep feces containing eggs of *H. contortus* on experimental plots.

A: feces in the form of pellets spread evenly over subplots; B: feces in the form of pellets piled in center of each subplot; C: pulverized feces spread evenly over subplots; D: pulverized feces piled in center of each subplot; E: feces reduced to fluid paste with an equal quantity of water and poured onto the center of each subplot.

larvae that failed to survive. The difference between the numbers of larvae recovered from the different subplots represents the total effect of the detrimental influences on the different subplots, and indicates that under natural conditions the destruction of eggs and larvae of the stomach worm is extensive. Concentration of fecal material or addition of water protects the preparasitic stages and allows larvae which otherwise would have perished to reach infectivity.

DISCUSSION

The three experiments reported in this paper were undertaken in order to secure information on the survival of eggs and preparasitic larvae of *Haemonchus contortus*

in feces, when exposed to conditions prevailing on grass plots. The first experiment showed that no larvae were recovered in the spring from sheep feces containing eggs or from grass surrounding feces deposited on grass plots during cold weather. As infective larvae can survive cold weather during the entire winter (Shorb, 1943) it follows that the contaminative material present was destroyed by cold when in the preinfective larval stages or as eggs. This being true, it likewise follows that eggs of *H. contortus* in any sheep feces which may be deposited on pasture during the late fall, winter, or early spring, will be destroyed by cold unless there should be an ensuing warm period sufficiently long for infective larvae to develop from the eggs. Such periods of mild temperatures are rare during the winter in temperate climates and, therefore, infected lambs or sheep can add little in the way of stomach worm contamination to the pasture during the cold weather, making pasture rotation during this period unnecessary.

The second experiment showed that summer sunshine and heat were highly lethal to eggs of feces not protected from weather. Although during the period of test much more than the normal amount of rain fell in the region where the work was done than was normal for the time of year, only 21 larvae were recovered from the bare ground, while about 5,000 larvae survived the same conditions when protected by grass 4 inches tall. It is not unreasonable to assume that even 21 larvae could not have survived had there been no rain or even if only the normal amount for the season had fallen. However, it is important to remember that even on bare ground a few larvae survived and reached the infective stage. These results seem to indicate that although overgrazing is a detriment to pastures, it contributes to the destruction of vast numbers of *Haemonchus* eggs and larvae by exposing them to sunlight. The reverse would likewise be true, that adequate grass cover favors survival of such eggs and larvae and emphasizes the need under good pasture conditions for rotation and treatment of infected animals.

When the feces from infected sheep were placed on grass plots and no additional water added, a certain proportion of the eggs survived and the resultant larvae developed to infectivity. By modifying (adding a quart of water daily to each 4 square feet of sod) 24 times as many larvae survived and were recovered from the grass. A smaller addition of water, whether applied daily or weekly, was reflected in a smaller increase in number of survivors. The increase in number of larvae surviving appeared to be due to the constant presence of water rather than to the increased amount. A modification in the consistency of the feces or especially in the concentration of fecal material also caused a modification of the number of eggs and larvae surviving until the infective stage was reached. Obviously the increase in number of infective larvae recovered from plots having feces in piles, or having feces reduced to paste by the addition of water, was due to conservation of moisture. One exception was noticeable in the subplot where the feces were scattered as pellets and the pellets received one quart of water daily. The reason for this exception is not known. The significance of this experiment, when the data are used for planning pasture usage, is in pointing out the danger of wet seasons and concentration of manure on pasture. The lambs should be so managed that there would be no undue concentration of feces around feeding or watering places, and they should be moved to clean pastures following unusually wet periods.

The effect of adding water to fecal cultures and to fecal material on grass plots presents an interesting contrast. As Veglia (1915) noted, and this observation has

been verified by other workers as well as by the present writer, the addition of water to fecal material in glass containers for the purpose of allowing larvae to develop actually caused the culture to fail if enough water was added to make the fecal material wet. On the other hand, the addition of water to the fecal material in the third experiment for the purpose of reducing the feces to a fluid paste not only failed to reduce the number of larvae which survived but actually favored their survival. This leads to the conclusion that if the essential difference between diarrheic feces and normal pellets is one of water content, diarrheic feces during the summer actually favor survival of preinfective eggs and larvae.

SUMMARY

1. No larvae of *H. contortus* were recovered from grass plots in the spring following the application of feces containing eggs at 4 different times during the previous winter.

2. Sunlight and heat were highly lethal to the second and third stage larvae of *H. contortus* exposed in feces on bare ground in August, in spite of heavy rains.

3. A 24-fold increase in larvae of *H. contortus* recovered from 2-foot square grass plots, compared to control plots, followed the daily application by means of a sprinkler of one quart of water, although both test and control plots received twice this amount of water in the form of rain during the 18 days of test. The difference in yield was undoubtedly due to the fact that the sprinkled water was more constantly present than that from rain. The concentration of feces conserved moisture present and this was reflected in the number of surviving larvae recovered.

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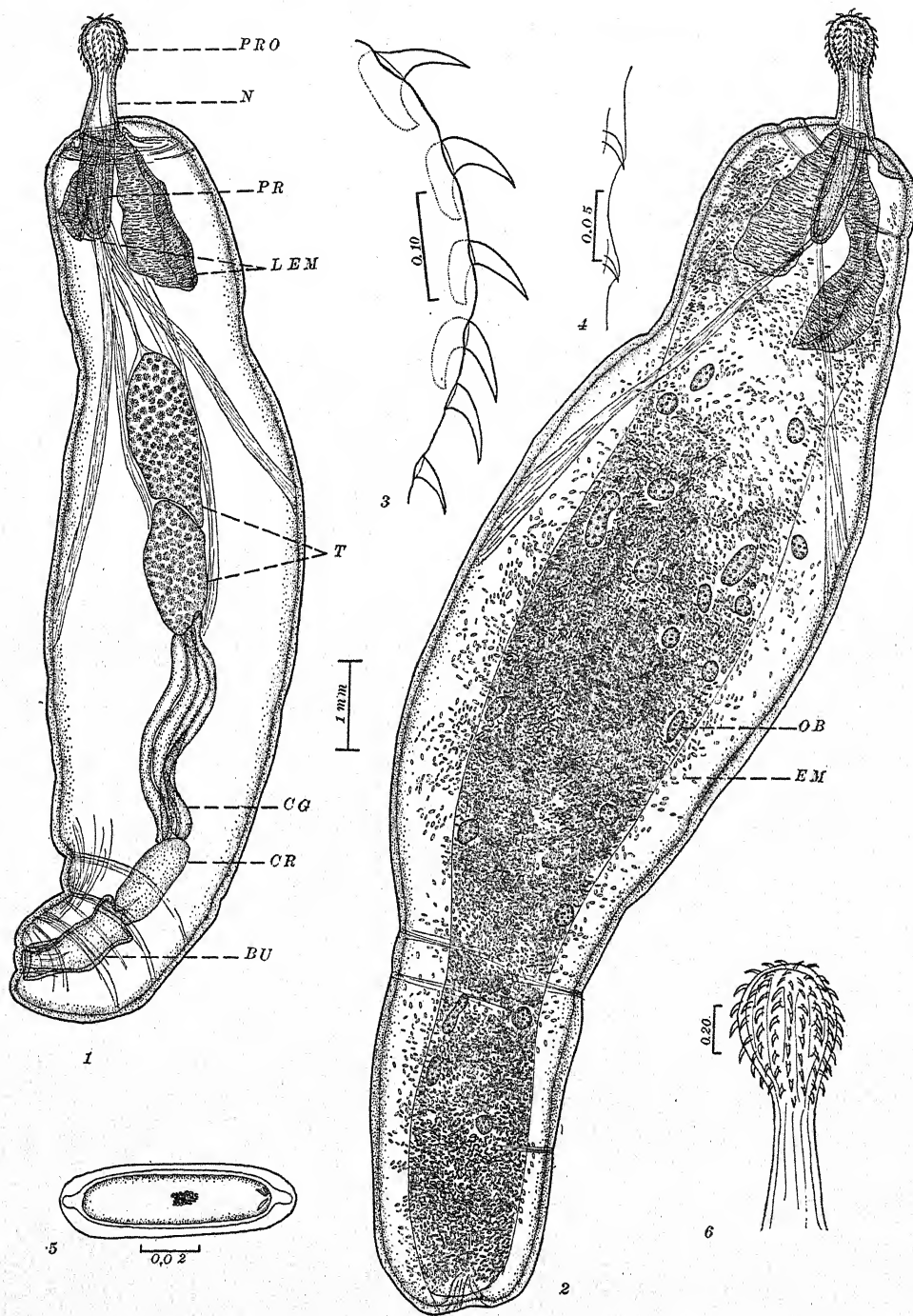
A REDESCRIPTION OF *POLYMORPHUS OBTUSUS* VAN CLEAVE, 1918 (ACANTHOCEPHALA)

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Van Cleave (1918) first described briefly the acanthocephalan, *Polymorphus obtusus*, from the water-turkey, *Anhinga anhinga*, and later (1925) identified as *P. obtusus* a specimen from the great blue heron, *Ardea herodias*. No subsequent description of this species has been found. Fourteen acanthocephala identified as *P. obtusus* have been recovered from the intestine of the ruddy duck, *Erismatura jamaicensis*. Since the writer has observed a number of morphological details not included in Van Cleave's papers, it seems desirable to extend the description of this species.

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*Polymorphus obtusus*

The writer acknowledges her indebtedness to Dr. R. M. Cable for the use of laboratory facilities of Purdue University.

Polymorphus obtusus Van Cleave, 1918
(Figs. 1-6)

Body cylindrical with constrictions near anterior and posterior ends; posterior end truncate. Mature males (Fig. 1) 8-12 mm long by 1-3 mm in maximum diameter; mature females (Fig. 2) 12-16 mm in length by 2-4 mm in maximum diameter. Proboscis (Fig. 6) elongate ovoid, 0.5-0.7 mm long by 0.26-0.40 in maximum diameter; armed with 14-16 longitudinal rows of 7-10 hooks each. Hooks at base of proboscis 0.049-0.062 mm long; other proboscis hooks 0.066-0.073 mm long with roots 0.066-0.079 mm in length. Neck attenuate, approximately 1.3 mm long and 0.3 mm wide. Anterior part of body covered with spines (Fig. 4) about 0.027 mm long, arranged in 30-40 longitudinal rows. Proboscis receptacle 1.12-1.54 mm in length and 0.24-0.39 in diameter. Lemnisci broad, fan-shaped, with irregular margins, and unequal in size, the larger being about twice as long as the proboscis receptacle. Testes of mature males oval, 0.77-1.5 mm long and 0.56-0.77 mm wide. The four cement glands are long and tubular, averaging 2.5 mm by 0.2 mm. Fully developed embryos (Fig. 5) of mature females 0.066-0.08 mm by 0.019-0.023 mm, with polar prolongations of the middle membrane.

The writer's specimens are considerably larger than those described by Van Cleave, show greater variation in the number of longitudinal rows of proboscis hooks, and have slightly larger body spines. These differences probably are not significant, since the two lots of material are from different hosts, and the writer's specimens, being more numerous than Van Cleave's, would be expected to show a greater range of variability.

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EXPLANATION OF PLATE

Drawings were made from stained whole mounts with the aid of a camera lucida. Figs. 1 and 2 are composite drawings.

- FIG. 1. Adult male.
FIG. 2. Adult female.
FIG. 3. Proboscis hooks from a single longitudinal row.
FIG. 4. Body spines.
FIG. 5. Embryo from a mature female.
FIG. 6. Proboscis of the specimen shown in Fig. 1.

BU—bursa
CG—cement glands
CR—cement receptacle
EM—embryo
LEM—lemnisci

N—neck
OB—ovarian ball
PR—proboscis receptacle
PRO—proboscis
T—testes

CULTIVATION OF *NYCTOTHERUS CORDIFORMIS*

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Attempts to cultivate *Nyctotherus cordiformis* heretofore have been unsuccessful. The conclusion has seemed justified that this organism cannot be grown in vitro using the methods and media ordinarily successful with intestinal protozoa; indeed the opinion was expressed by Wichterman (1936) that *Nyctotherus cordiformis* is anaerobic. In the present study a method was found whereby this organism can be cultivated in an ordinary medium and without the application of anaerobic technique. The cultivation method employed is an adaptation of an intestinal content medium method previously devised (Nelson, 1940) for the cultivation of *Balantidium coli*.

MATERIALS AND METHODS

Adult Leopard frogs, *Rana pipiens*, caught in the field or obtained from supply houses were used as the source of *Nyctotherus* throughout this study.

Preliminary attempts to cultivate *Nyctotherus* gave very encouraging results and provided a basis for the technique finally employed. In these preliminary trials the rectal contents of a frog infected with *Nyctotherus* were removed as a lump from the rectum and dropped into 10 ml of frog-Ringer's solution in a test tube. A small mass of rice starch was dropped at the side of the lump of rectal material. Careful examination with a hand lens showed that in about an hour the ciliates began to emerge from the rectal material, congregate at the starch mass, feed and then disperse. They did not return to the rectal material but migrated outward along the inside surface of the tube. For the most part the organisms spent their time crawling around on the surface of the glass.

In crude cultures of this type the ciliates lived for over a month. Morphologically and physiologically they appeared perfectly normal and some multiplication took place.

Preparatory to additional experiments an assembly was devised to (1) prepare rectal content cultivation medium, (2) separate the nyctotheri from the mass of rectal material and (3) permit removal of the mass of rectal debris from the cultivation tube. This "extraction assembly" is shown in Fig. 1. Sterile frog-Ringer (NaCl 6.5 gm, NaHCO₃ 0.20 gm, KCl 0.14 gm, anhydrous CaCl₂ 0.12 gm, NaH₂PO₄ 0.01 gm, H₂O 1000 ml) was added to a depth of about 3 cm above the absorbent cotton in the extractor before inoculation.

Inoculation and operation of the assembly is as follows: a frog is opened, the rectum removed and the contents taken up in a large-bore pipette and introduced into the extractor over the cotton. The rectal material gives off fluid and fine solid material which diffuses and settles through the cotton into the fluid below to produce rectal content cultivation medium. The nyctotheri in the rectal material migrate downward through the cotton to the bottom of the test tube. Their movements can be followed readily with a 10× hand lens. The left-over rectal debris remains in the extractor above the cotton and when desired is removed by simply lifting out

the extractor. There remains the test-tube of debris-free rectal content cultivation medium in which to continue cultivation of the nyctotheri obtained.

RESULTS

The first experimental series was run at the Mt. Desert Island Biological Laboratory in Maine.

On July 16 an extraction culture was inoculated with the rectal contents of a small specimen of *Rana pipiens* caught in a near-by marsh. Within a few minutes after

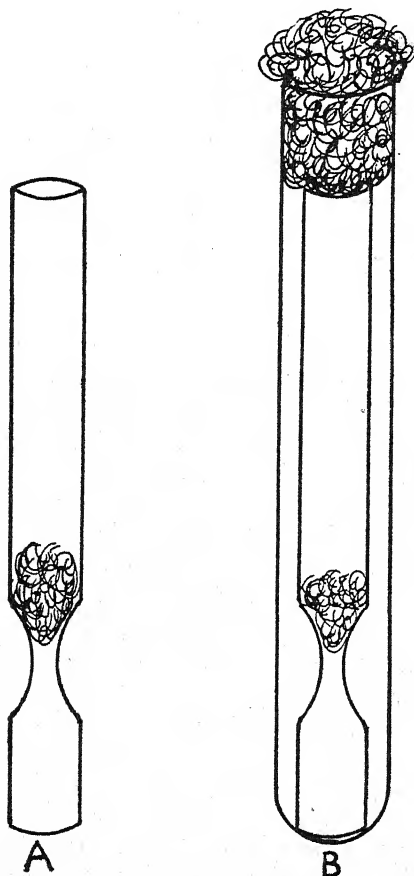


FIG. 1. Extraction assembly. The extractor tube (A) is prepared from glass tubing with a thin cotton plug placed above the constriction. This tube is introduced into a test tube of medium to produce the extraction assembly (B). Material from which it is desired to separate protozoa is introduced into the extractor tube over the cotton plug. The protozoa migrate through the cotton to the bottom of the test tube. When desired, the extractor with the unwanted material can be lifted out.

the introduction of the inoculum nyctotheri were observed migrating into the cotton plug. For 45 minutes they remained in close proximity to the inoculum during which time visible downward diffusion and sedimentation from the inoculum progressed, and then they continued their migration to the bottom of the tube.

The subsequent history of the culture is as follows: at nine hours the nyctotheri were congregated at the bottom in an actively moving swarm. A small amount of

rice starch was fed and within twenty minutes the organisms became engorged and the swarm dispersed. Instead of swimming freely the ciliate was observed to be, habitually, a crawler. It crawled up the inside of the tube in a peculiar hypotrich-like manner and was able to cling with such tenacity as not to be dislodged through ordinary handling of the tubes. A count with a hand lens showed that there were about sixty-five nyctotheri in the culture.

At 22 hours the population had increased to about 120 and a number of dividing individuals were seen. Although most of the ciliates were crawling on the surface of the glass near the starch, many had crawled up the inside of the test tube, some to within a centimeter of the surface of the liquid.

At 48 hours the extractor tube with the inoculum was transferred to a new tube of Ringer's to produce a second tube of medium (Culture No. 2). Some nyctotheri clinging to the extractor accompanied the transfer. About 160 organisms remained in Culture No. 1. During the rest of the life of the culture starch was fed every three or four days.

At 10 days the population in Culture No. 1 had reached about 280 after which there appeared to be little or no increase, although the organisms apparently remained normal in appearance and activity.

On the 30th day the culture suddenly succumbed to a strange great bacterial growth following a starch feeding.

Culture No. 2 was given a slightly different treatment. At ten hours the extractor, with its contents and some clinging nyctotheri, was shifted to a new tube of Ringer's solution (Culture No. 3). About 40 nyctotheri remained in Culture No. 2. This culture was maintained for 54 days during which numbers of nyctotheri were removed for use in other experiments. The population at intervals and the numbers removed were as follows:

115 on	3rd day,	30 removed
180 on	5th day,	50 removed
420 on	30th day,	100 removed
370 on	35th day,	50 removed
400 on	38th day,	100 removed
480 on	44th day,	80 removed
420 on	54th day,	100 removed

This was a very healthy culture throughout the period of observation but it had to be terminated on the 54th day.

Culture No. 3 did not respond as well as the first two and this seems to have been due to the poor quality of the medium, as the beneficial extracts in the inoculum had been largely removed in the first two cultures.

The culture started with a population of 32 and increased slowly to about 140 on the 8th day. About 60 nyctotheri were removed on this day for use in other experiments. The loss was never entirely made up and after the 34th day the culture declined, and no living nyctotheri were seen after the 52nd day.

A second experimental series was run during the next winter in the laboratory at the University of Maine. The inoculum was taken from frogs purchased from a supply house.

On February 8th an extractor was inoculated with the rectal contents of a large frog. At the end of 24 hours approximately 60 nyctotheri were found to have descended from the inoculum. Starch feedings were given every four or five days during the life of the culture. The extractor tube was left in during the whole period. The population increased slowly to about 200 on the 15th day, about 250 on the 40th day and to a peak of about 300 on the 66th day. Thereafter a decline set in and by the 80th day the number had dropped to about 60. Only 12 active organisms and many cysts were found on the 101st day. A small amount of distilled water was added to make up for the evaporation but did not revive the culture and on and after the 105th day no active organisms were found.

A second extraction culture of Ringer's solution was inoculated on Feb. 9th with the rectal contents of a frog which had died of "red leg." At the end of 24 hours 74 nyctotheri were found to have descended from the extractor tube. The extractor tube with the inoculum was removed on the sixth day. As in the previous instances multiplication progressed slowly and steadily and by the 40th day about 270 nyctotheri were present. A slight decrease was noticed from then to the 47th day. To make up for water evaporation a small amount of distilled water was added. This had a marked stimulating effect and by the 51st day a sharp increase to about 340 organisms took place. After this date encystation began and the population dropped steadily. On the 100th day only 44 active ciliates were present. A small amount of distilled water was added. On the 105th day only three active ciliates were present and none were seen on and after the 111th day. A dilution count showed about 600 cysts to be present in the culture. These cysts appeared perfectly normal.

A third extraction culture inoculated on March 27th gave essentially the same results as the others. The extractor tube and inoculum were removed on the seventh day. Starch feeding was delayed until the seventh day by which time most of the nyctotheri had starved to death. The remaining organisms responded immediately to the starch feeding and a vigorous culture resulted. From an initial population of 23, the colony increased to 130 in 11 days, to 370 in 32 days and remained at approximately this level until the 53rd day when the population began to decline. Only 29 organisms were present on the 71st day and none on the 76th day. Excessive water loss from evaporation may have accounted for the shortened life of this culture.

Cultivation attempts in plain Ringer's solution.—Growth in plain Ringer's solution was attempted to determine to what extent the success of the extractor method was due to extracted material from the inoculum. Eight trials were made. In each, 15 to 40 nyctotheri were transferred to test tubes of Ringer's solution and starch was given. No multiplication took place and the organisms lived only two to four days.

Cultivation attempts in frog blood-Ringer's solution.—Attempts to grow the organisms in frog blood-Ringer's solution proved almost equally unsuccessful. In three attempts tubes of the medium were inoculated with from 50 to 100 organisms. A number of reinoculations were made when the organisms were found dead in two or three days. In one instance the ciliates lived for 22 days but showed lack of vigor and no apparent multiplication.

Cultivation in pig cecal content medium.—In pig cecal content medium (Nelson, 1940) *Nyctotherus* was cultivated for periods up to 86 days in individual tubes and by transfer for a total of 157 days, when the culture was voluntarily terminated. Cul-

ture No. 1, a test tube of 10 ml of three-month-old pig cecal medium, was inoculated with 50 *nyctotheri* from a 23 day old extraction culture. Growth and multiplication proceeded without delay. The population increased to about 400 by the 20th day and then slowly to a peak of about 550 on the 61st day. A decline then set in, possibly due to excessive loss from evaporation. By the 76th day the population had dropped to 56 and after the 86th day no live organisms were seen.

A subculture of 6 *nyctotheri* in a tube of the same medium was made on the 76th day. In three days the population had increased to 13. At the end of 23 days the population had increased to about 180 after which it increased slowly to about 300 by the 68th day. Thereafter the population began to decline with most of the organisms apparently encysting and after the 81st day no active organisms were seen.

DISCUSSION

The writer has been unable to reconcile the statement that *Nyctotherus* is anaerobic (Wichterman, 1937, p. 567) with the results obtained in this study. No apparent need for anaerobic conditions was noted; in fact the organism was not loath to ascend to the region near the exposed surface of the culture medium and did so without suffering any adverse effect. Of course the unsuccessful cultivation attempts or other data on which Wichterman has based his conclusions are not strictly comparable. He obtained his *Nyctotherus* from *Hyla versicolor* and in the present study *Nyctotherus* from *Rana pipiens* was used. It remains to be seen whether the difference in origin may account for the difference in results. As far as is known at the present time the same species of *Nyctotherus* is present in both frogs.

The ordinary criteria of successful cultivation were satisfactorily fulfilled in the present study. Feeding, growth and normal multiplication took place and morphologically and physiologically the ciliates appeared normal. The continuous cultivation period of any single strain was only 180 days (with subcultures on the 23rd and 76th days) but there was good assurance that cultivation could have been continued indefinitely. Individual cultures lived as long as 105 days.

Opportunity was not afforded for detailed observations to determine to what extent the whole life-cycle as outlined by Wichterman (1937) was completed. Wichterman found that in the host, conjugation of *Nyctotherus* takes place only during the period of transformation of the tadpole to the frog, a period of critical changes in the animal and in the intestinal environment of *Nyctotherus*. Cultivation of *Nyctotherus* provides an opportunity to investigate in vitro the factors involved in this interesting phenomenon.

Certain of the basic requirements of the organism became apparent during this study. In plain Ringer's solution the organism does not grow or multiply and it dies in a relatively short period. The addition of rectal content extract converts Ringer's solution into a medium in which life proceeds normally until the organism dies from starvation. Starch added to this medium adequately meets nutritional requirements to promote growth and multiplication. Of the basic unknown supplements so well provided by the intestinal content extract in the medium, the bacterial population may be of critical importance in the cultivation of *Nyctotherus* as was found to be true in the case of *Balantidium* (Nelson, 1940). Serum supplement is not needed in the medium.

The successful growth of *Nyctotherus* obtained in the tests made with pig cecal content medium would seem to indicate a similarity in the requirements of *Nyctotherus* and *Balantidium*. Since both pig and frog supply, in their intestinal content, constituents essential to the growth of *Nyctotherus* it is possible that the constituents are the same in both instances. The possibility suggests itself that *Nyctotherus* could live in an animal like the pig if the temperature difference could be overcome. A number of unconfirmed reports of *Nyctotherus* infection in man and other warm blooded animals have been published. Conversely there are a number of species of *Balantidium* described from frogs.

By comparison with ordinary standards of multiplication rate and population peaks in cultures of parasitic protozoa, *Nyctotherus* is disappointing. Multiplication is deliberate, apparently amounting to only one or two divisions per 24 hours in the fresh culture and dropping steadily below this as the population increases and the culture ages. The population peak reached in a culture was about 550 nyctotheri. The total production of cultures from which organisms were withdrawn at intervals considerably exceeded this peak number.

Encystment, a rather infrequent occurrence in cultures of parasitic protozoa, took place in some of these cultures as they aged.

SUMMARY

1. *Nyctotherus cordiformis* from *Rana pipiens*, apparently not hitherto successfully grown in vitro, has been kept under continuous cultivation for periods up to 180 days and then terminated voluntarily with good assurance that cultivation could have been continued indefinitely. Individual cultures lived as long as 105 days.

2. The organism was grown in Ringer-rectal content extract medium and fed with starch. Serum supplement to the medium was not needed. Ordinary cultivation methods were used and no indication was found that the organism needs anaerobic conditions.

3. A method designated as the "extraction culture" method was devised for separating the organisms from the rectal contents and at the same time preparing a tube of the medium.

4. The usual criteria of successful cultivation were satisfactorily fulfilled. Feeding, growth and multiplication proceeded normally and the ciliates appeared normal morphologically and physiologically. The organism seems to multiply slowly and the peak population reached in a culture was about 550. Encystment took place in some cultures as they aged.

5. Cultivation was successfully obtained in pig cecal content medium also. *Nyctotherus* appears to be similar to *Balantidium* in its requirements.

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RESEARCH NOTES

THE PREVALENCE OF PINWORM INFECTION IN AN OHIO INSTITUTION FOR CHILDREN

Many surveys have been made to determine the prevalence of infection with *Enterobius vermicularis* in both institutionalized and non-institutionalized groups. The more recent surveys in which use has been made of special diagnostic methods related to the characteristic ovipositing habits of the gravid female have shown that the prevalence of infection is much higher than formerly believed. Recently, the results of many surveys as well as other literature pertaining to the parasite have been summarized adequately by Cram (1941, An Introduction to Nematology, edited by J. R. Christie, Sec. II, Part II: 322-324). The present report is submitted as an addition to the existing data on the prevalence of oxyuriasis in institutionalized groups in the United States.

From April 4 to May 16, 1939, anal swab examinations were made on a group of institutionalized white children near Alliance, Stark County, Ohio. The group included both males and females, ranging in age from 3 to 18 years. Most of the children had been in the institution for more than three months, so that little effort was made to ascertain from what type of home each individual had come. The results of the examinations are given in the accompanying table.

In general, the living conditions in the institution were good. The children were divided by sex and roughly by age into six groups, each of which lived in a separate dormitory. All buildings were clean and sanitary facilities were excellent. The children slept in separate beds; however, daily bathing was not required, and the clothing was usually worn for several days before being laundered. It was possible, therefore, that ova might be carried about for several days on the bodies and in the clothing of the children. This condition probably favored the maintenance and spread of the infection.

The NIH swab devised by Hall (1937, Am. J. Trop. Med. 17: 445-453) was used throughout this survey and the finding of a single ovum on the swab was considered indicative of infection. Because of the institutional routine, most of the examinations had to be made after the morning meal. Certain infected individuals may have removed the ova from the perianal region by defecation or bathing before the examination was made, so that the number of positives may have been slightly higher than shown by the examinations. Those individuals who were positive by the first swab series were not re-examined. Of the 192 individuals who were negative by the first swab series, 125 were examined a second time.

Of the 158 males examined, 72 or 46% were infected and of 111 females examined, 34 or 31% were positive. This difference is largely due to high percentage of infected males in dormitory 3 (75%). Grouped arbitrarily by age, the rates of infection were as follows: 3-4 years, 50% (2 out of 4); 5-9 years, 65% (39 out of 60); 10-14 years, 41% (55 out of 133); and 15-18 years, 14% (10 out of 72).

TABLE 1.—NIH swab examinations of 269 institutionalized white children

Dormitory and age	Sex	No. exam. once	No. pos. 1st swab	No. exam. twice	No. pos. 2nd swab	Total no. exam.	Total no. pos.	% pos.
No. 1 13-18	M	37	5	0	0	37	5	14
No. 2 12-15	M	42	12	30	4	42	16	38
No. 3 8-13	M	48	25	22	11	48	36	75
No. 4 3-9	M	31	7	23	8	31	15	48
No. 5 4-13	F	51	18	32	4	51	22	43
No. 6 12-18	F	60	10	18	2	60	12	20
Total		269	77	125	29	269	106	39

Of the 269 children examined, 77 or 29% were determined positive by the first swab series (Table 1). Of the 125 individuals who could be examined a second time, 29 were found to be positive. Thus, 106 or 39% of the 269 children examined were found infected after the second swab series. This increase in the number of infections determined by using repeated swab

series has been noted by several investigators. It seems likely that more infected individuals would have been determined in this institution if additional swab series could have been made.

The figures in Table 1 show a lower incidence of infection in the children examined from dormitories 1 and 6. This difference may be partially explained on the basis of the number of positive individuals determined by second examinations. Of 110 individuals in dormitories 2, 3, 4, and 5 who were negative by the first examination, 107 were examined a second time, whereas of 80 individuals in dormitories 1 and 6 who were negative by the first examination, only 18 could be examined a second time.

The percentage infection (39) of this group of institutionalized children is considerably lower than the average percentage infection (63) for institutionalized persons of six localities in North America as summarized by Cram (loc. cit., Table 11). This difference may be explained partially on the basis of the number of swab series used in making the survey, since in most of the surveys summarized by Cram 4 to 7 swabs per person were used as compared with 2 per person in this study.—ALAN W. DONALDSON, *Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.*

A LEECH FEEDING ON LIGULA

On July 16, 1941, a leech was discovered in the act of feeding on a live ligula along the shore of Wolf Lake, Huntington Forest property of the Roosevelt Wildlife Forest Experiment Station, Newcomb, N. Y. When placed in formalin the leech endeavored to disgorge the ligula, but succeeded in releasing only a small portion before succumbing. The uneaten portion of the worm was 160 mm long and 4 mm wide (see Fig. 1).

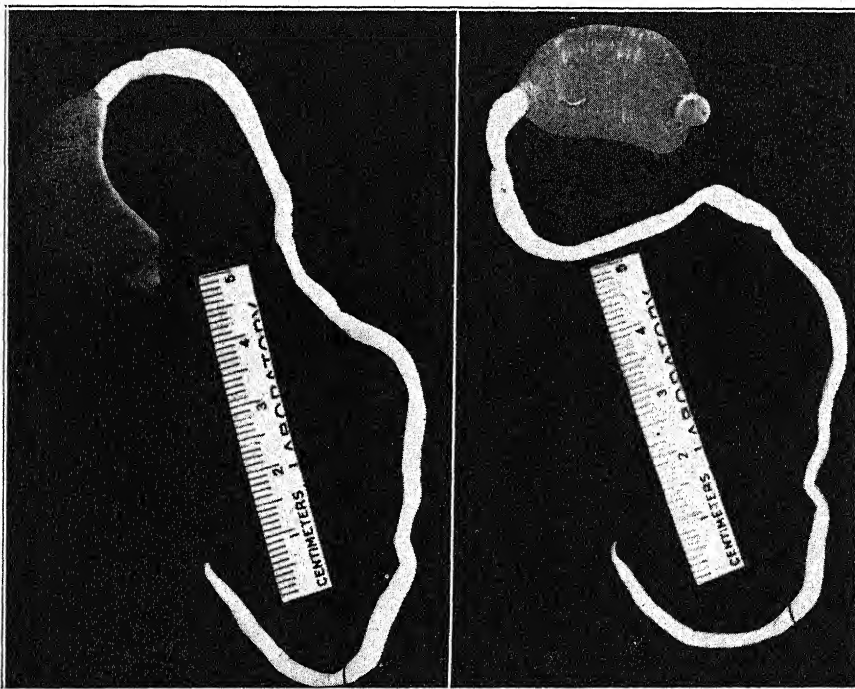


FIG. 1. Leech feeding on ligula. Dorsal view at left, ventral view at right. Photograph by J. F. Mueller.

Ligulas are sometimes found free and alive in the shallow shore areas of at least two lakes on the Huntington Forest (Dence, 1940, Copeia, 2: 140), where about one-fourth of the large population of common shiners (*Notropis cornutus*) are infected with ligulas. Badly infected individuals, with distended bellies, commonly frequent the shallow sandy shore areas throughout the summer. It is presumed that some of the parasites are liberated by the predators, such as mergansers and herons, during the feeding process, although there is evidence that worms are

occasionally freed by other means also. The ligula that was attacked by the leech was one of several at the mouth of a little inlet.

This appears to be the first record of a leech feeding on ligula, probably because free ligulas are never very abundant. Leeches were not abundant in Wolf Lake either. Many of our fresh water leeches are predacious and regularly feed on small aquatic animals.

The species of leech has not been positively determined, but Dr. J. Percy Moore, after examining photographs, stated that it was undoubtedly a species of *Haemopis*, probably *marmoratis*, but possibly *grandis*.—WILFORD A. DENCE, *New York State College of Forestry, Syracuse, New York*.

SARCOCYSTIS RILEYI (STILES, 1893) IN THE DOMESTIC FOWL,
GALLUS GALLUS

Sarcocystis sp. has been reported from twenty-one species of birds, eleven in the United States (Erickson, 1940, *Auk* 57: 514). However there have been few reports of its presence in the domestic fowl, *Gallus gallus*. The records in domestic fowl are: *Sarcocystis* sp. Stiles, 1893 (U. S. Dept. Agric. Bur. Animal Industry, Bull. 3: 79-88) and Stiles, 1894 (Vet. Mag. Phila. 1: 728), both in the United States; *Sarcocystis howarthi* v. Ratz, 1908 (Allattani Kozlemenyek 7: 177) in Hungary; and *Sarcocystis gallinarum* Krause and Goranoff, 1933 (Z. Infektionskr. und Haustiere 43: 261-279) in Bulgaria.

Recently we noted the presence of Sarcosporidia in domestic fowl from North Adams, Michigan. The bird was submitted to the poultry clinic of this institution for diagnosis. Sarcocysts were noted on the external surface in the muscles of each thigh. Sections revealed a light infection in several muscle bundles. This specimen did not differ sufficiently from those of *Sarcocystis rileyi* from the mallard (*Anas platyrhynchos platyrhynchos*), the domestic mallard (*Anas platyrhynchos domestica*), or the black duck (*Anas rubripes*) in our possession to justify a new species.

It is apparent that *S. howarthi* v. Ratz, 1908, and *S. gallinarum* Krause and Goranoff, 1933, are synonymous with *S. rileyi* (Stiles, 1893).—PHILIP A. HAWKINS, *Department of Bacteriology and Hygiene, Michigan State College*.

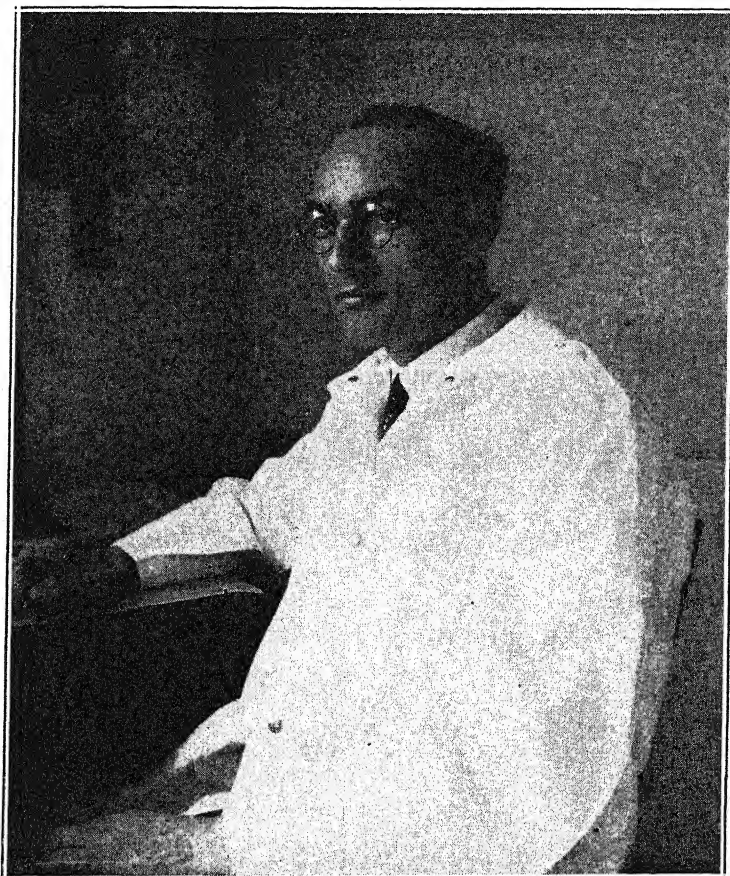
THE ORIENTAL RAT FLEA (XENOPSYLLA CHEOPIS) IN MICHIGAN

On November 14, 1942, a wild house-rat (*Rattus norvegicus*) was killed in the animal house of the Zoology Department of the University of Michigan. Approximately one hundred specimens of the Oriental rat flea (*Xenopsylla cheopis*) were recovered.

Since 1925 when Wallace first reported this flea in Indianapolis, this medically important insect has been found in many of the central states including Indiana, Illinois, and Ohio. I have been unable to find a report of *Xenopsylla* from Michigan and consequently thought it advisable to record its expanding range.—LEO JACROWSKI, JR., *Zoology Department, University of Michigan*.

IN MEMORIAM

WILLIAM ALBERT HOFFMAN (1894-1943)



Wm. A. Hoffman

Dr. William A. Hoffman, Head of the Department of Medical Zoology, School of Tropical Medicine, San Juan, Puerto Rico, died on April 5, 1943. His death was a great shock to all his friends, for it ended prematurely an active and useful life which was remarkable for the overcoming of handicaps resulting from an attack of poliomyelitis at an early age.

Dr. Hoffman was born in Long Branch, New Jersey, on April 18, 1894. He received his B.S. from Cornell University in 1917 and his Sc.D. from the Johns Hopkins School of Hygiene and Public Health in 1924. The following year he was medical entomologist of the Haiti Survey for the International Health Division of

the Rockefeller Foundation, and in 1926 he joined the staff of the School of Tropical Medicine in Puerto Rico as parasitologist, a position which he held until his death.

At the Johns Hopkins University he was an assistant of the late Dr. F. M. Root, under whose guidance he completed his thesis for the doctorate on the *Culicoides* of North and Central America and the West Indies. This review included descriptions of 21 species, 6 of them new, and in addition, two new varieties. Primarily an entomologist, Dr. Hoffman maintained a deep interest in these insects, and came to be recognized as a leading authority on the biting midges in the New World. He continued to describe new species, but most of his efforts in this field were devoted to a cooperative project with Dr. Root on a monograph of the American *Culicoides*. Interrupted by Dr. Root's death, this work nevertheless resulted in a revision of the North American species of *Culicoides*, which included descriptions of 13 new species. At the time of his death, Dr. Hoffman was in possession of a considerable amount of unpublished data on biting midges and a large collection. Fortunately, his collection will be placed in the U. S. National Museum, where it will be available for further study. He was also well acquainted with the mosquitoes and had made several surveys in the Caribbean region.

His interests by no means were limited to entomology. His contact with tropical diseases led to many other investigations. In a series of papers written in part with Dr. E. C. Faust and others, on schistosomiasis, the distribution of *Schistosoma mansoni* in Puerto Rico was established, the intermediate host determined, and details of biology described. A sedimentation-concentration method for diagnosis of infections was devised, the use of which was urged as a means of preventing the introduction of the parasite into regions where it is not yet present. Unpublished results of his investigations on schistosomiasis included observations on pathology, and on the epidemiology and methods of control in Puerto Rico. Several papers were also written on *Fasciola hepatica*. The intermediate host was found and a precipitin test was prepared for diagnosing infections with this parasite. Finally, a survey of filariasis in Puerto Rico showed the incidence and distribution of *Wuchereria bancrofti* in the island.

Dr. Hoffman could walk only with the aid of special braces. In spite of this difficulty, in strenuous field trips he could set a stiff pace for many a younger and healthy collector by his enthusiasm and energy. He would refuse, in fact, resent any offer of aid, and his determination to equal or surpass the endeavors of people more favored physically, combined with his careful observations and sound reasoning, made him the successful scientist he aspired to be. Psychologically, he fought an uphill battle all his life. His deep love for fine music was a tremendous aid in this fight; many times he remarked that in periods of depression he could change his mood by playing his beloved violin or viola.

Parasitologists and entomologists will remember him through his contributions to their fields; his more intimate associates will remember him also as a kind and loyal friend.—L. E. ROZEBOOM, *School of Hygiene and Public Health, Johns Hopkins University*.

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OBSERVATIONS ON HOST-PARASITE RELATIONS BETWEEN LABORATORY MICE AND *NEMATOSPIROIDES* *DUBIUS* BAYLIS*

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The writer undertook a series of infection experiments using highly inbred strains of laboratory mice and *Nematospiroides dubius* Baylis. This nematode (Family HELIGMOSOMIDAE) is a normal parasite of wild house mice. The experiments were conducted through 1940 and the spring of 1941 at Davis, California.

MATERIALS AND METHODS

Three strains of mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. These strains were all highly inbred by brother-sister matings so that a high degree of homozygosity could be expected in each. There were no visible size differences among the animals of each strain. The strains consisted of the following:

(1) C-57 stock, inbred 20 generations; black in color; of genetic formula *aa*; characterized by mammary carcinoma in about 1% of the breeding females, none in virgins; various types of internal tumors, mostly sarcomas, 10-20% in both sexes, high fertility;

(2) A-W stock, white-bellied agouti, inbred 20 generations, genetic formula *A^wA^w*; medium fertility;

(3) L stock, inbred 40 generations, non-agouti brown leaden, genetic formula *aabbd2d2*; derived by mutation from C-57 br (which was derived by mutation from the C-57 strain) of medium fertility.

According to Babcock and Clausen (1927) matings of brother and full sister represent the most effective method of promoting homozygosity in animals. Their Fig. 195 (adapted from Wright) shows about 98 per cent homozygosity in all characters to be attained after 16 generations of brother-sister matings. On this premise the strains used in the present experiments should be 98 per cent or more homozygous in all genetic characters, since all derive from brother-sister matings through at least 20 generations. Mice have 36 chromosomes. These mice, after their arrival on August 12, 1939, were mated at random within each strain in order to multiply their numbers, and this practice was followed in all subsequent generations.

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* The writer is deeply indebted to Dr. M. A. Stewart, Dr. W. B. Herms and Dr. S. F. Light of the University of California, who have generously contributed criticisms and suggestions and to Dr. P. W. Gregory of the same institution, for advice on the genetic aspects of the investigation.

The mice were housed in screen-top galvanized pans provided with sawdust and shavings. Water was offered in suspended bottles.

The diet consisted of dog chow in large checkers (presumed to be an adequate diet), and supplemented by one feeding of carrots and one of alfalfa alternating at weekly intervals.

Infected animals were housed and fed as was the normal colony, except that the litter was changed twice weekly to guard against completion of the parasite life cycle. Uninfected control mice were caged with test animals throughout all experiments. The cages were cleaned with a metal scraper and then rinsed in 2% lysol solution as a further safeguard against survival of infective larvae. It was previously determined that infective larvae are killed by exposure to 2% lysol for one minute.

The original stock of *Nematospiroides dubius* used in these experiments was taken from the small intestines of wild house mice (*Mus musculus musculus* Linnaeus) collected November 25, 1939, in a rice field on the Conway Ranch near Davis, California.

The life cycle of *N. dubius* will be described in detail in another paper. The following summary outlines the features essential to understanding the experiments to be described.

Eggs of *Nematospiroides dubius* are carried out in the feces of the host, hatch, and pass through the free-living larval stages in moist fecal material. The sheathed infective larvae migrate to the surface of the fecal mass by the 4th to 6th day after fecal deposition. Such larvae upon being ingested by a host mouse, molt while in the lumen of the intestine, then migrate through the mucosa, and come to lie in loose cysts in the circular and longitudinal muscle layers of the intestinal wall. There the last molt occurs, the young adult worms returning to the lumen of the intestine, where mating and oviposition take place. The entire cycle, from egg to egg, is completed in 13 to 15 days in the A-W strain of mice and fecal counts may be positive from 7 to 8 months following infection.

Larvae were washed from fecal cultures on the fifth or sixth days from the time of culturing, rinsed twice, and left in clean tap water.

Infection was accomplished by administering a counted number of infective larvae with a small pipette to mice deprived of drinking water for 24 hours. The pipette was next filled with clean tap water, also given to the mouse, then the pipette was rinsed and the rinse water checked beneath a binocular dissecting microscope to be certain that all of the larvae had been administered. Restraint of the mice while being infected was unnecessary; being thirsty, they licked avidly at the end of the pipette.

The larvae used in experimental infections were all from cultures of 6 to 15 days of age. These age limits were used to avoid any effects of age in different cultures of larvae. The same procedure was followed by Africa (1931) in his experiments with *Nippostrongylus muris*.

To obtain autopsy data, the mice were sacrificed, the viscera removed, and the small and large intestines were slit open. Any worms present were removed under a binocular dissecting microscope. The live worms were kept in 0.7% NaCl overnight, and counted the next day under the dissecting microscope, then preserved in 10% formalin.

In the experiments on acquired resistance to superinfection the worm measure-

ments were made by outlining the length of the worm under a camera lucida and measuring it with a calibrated wheel.

MORTALITY EXPERIMENTS

Fecal examinations were conducted on 32-day-old mice of the L, A-W, and C-57 stocks after infection with 100 larvae each of *Nematospiroides dubius*. The egg count data, however, are inconclusive and the writer feels that larger numbers of animals should be egg-counted before entirely reliable data can be obtained. In his experiments only 6-10 test animals plus 1 control could be egg-counted in each strain.

In the A-W strain of mice mortality to the dosage of 100 larvae was noted during the egg-count period while none occurred in other strains. Consequently further infection experiments were set up in which 32-day-old mice were infected with 100 larvae each of *N. dubius*. In addition one group of 26 A-W mice 32 days old were given 500 larvae each. Controls were run to equal half the number of test animals within each group and at the end of each experiment were either autopsied or egg-

TABLE 1.—Mortality percentages

Strain of mice	No. of mice	Larvae given	% mortality
L	19	100	0
C-57	25	100	0
A-W	50	100	26
A-W	26	500	100
Control mice same age	Approx. 50% of each group	0	0

counted 3 times to be sure that infection had not occurred in them. The results are given in Table 1. The 50 A-W mice given 100 larvae each showed a 26% mortality with the deaths occurring from 15-35 days after infection and the 26 A-W mice given 500 larvae each showed 100% mortality with deaths 8-23 days after infection. No further deaths were observed in either group up to the 50th day after infection or in the other strains or controls for the entire period.

Using mortality as a criterion the author concludes therefore that the A-W strain is more susceptible to the infection of 100 larvae than are the L and C-57 strains.

Symptomatology.—A-W mice were the only ones showing symptoms of infection. These began to weaken 1-10 days before death. Progressive emaciation, sensitivity to cold and roughness of coat were always present. The mice showed a reddish-brown to watery diarrhea, the feces often forming a large anal plug at death. Appetite was retained in some instances up to within a few minutes of death, which occurred on the 16th to 35th day following infection.

Post-mortem findings.—Lungs were normal. There was a slight serous to sero-sanguinous exudate in the body cavity; liver and kidneys were light colored, sometimes bleached or pallid; the spleen was often enlarged and light pink; the small intestine was enlarged, especially in its anterior 2 inches, fragile, light in color, and translucent. Appearance of the abdominal cavity was indicative of a severe anemia.

The stomach, small and large intestines were filled with food. The intestinal lumen often contained blood clots, especially in animals dying early in the infection period; the mucosa was severely inflamed or with heavy petechial hemorrhage, particularly about the heaviest concentrations of worms; the latter were usually grouped anteriorly in duodenum but in more chronic cases were scattered throughout the

tract, and in few instances, were observed passing out through the anus in large numbers. There was perforation of the anterior duodenum in severe cases with death from peritonitis. The large intestine and cecum were filled with feces, usually of a dark chocolate color and giving positive benzidine test for blood. The anus was often blocked with a firm, dried fecal plug.

STRAIN COMPARISON BY INTERVAL AUTOPSIES

Infection Experiments in 32-day-old Mice

A-W and C-57 mice were infected at the age of 32 days with 500 larvae each. Autopsies were conducted 7, 11, 18, 25, and 32 days respectively following infection. The average numbers of worms recovered from each strain of mice at the various autopsy intervals are given in Table 2. None of the A-W mice survived up to the 18th day following infection, whereas the C-57 mice showed no mortality up to the 32nd day following infection, when the last of the latter were sacrificed. There is no significant difference between the two strains of mice as to the number of worms recovered. In Table 3 the worm populations of the animals in Table 2 for the 7th

TABLE 2.—Total worm recovery averages with probable error of the mean; dosage was 500 larvae per mouse; mice 32 days old

Days after infection	7	11	18	25	32
A-W strain, 5 mice per period	478 ± 2.5	478 ± 3.1	*	*	*
C-57 strain, 5 mice per period	444 ± 2.8	456 ± 2.1	451 ± 3.7	459 ± 3.6	406 ± 26.8

* 30 more A-W mice died of the infection within 18 days after infection. The worm population in these animals was not ascertained.

TABLE 3.—Average worm distribution from Table 2

Days after infection	7		11	
Worm recovery percentages	From cysts	From lumen	From cysts	From lumen
A-W strain	87 ± 0.7	13 ± 0.7	*	100
C-57 strain	74 ± 0.5	26 ± 0.5	*	100

* Less than 0.1.

and 11th days have been broken down to show the distribution of their occurrence within the host. Since a larger percentage of the worms are able to pass through the encysted stage on the intestinal wall by the 7th day in the C-57 as compared to the A-W strain it is apparent that there is some factor or factors operating in one of the other of these strains which either speeds up the encysted phase of the life cycle in the C-57 strain or slows it down in the A-W mice. This factor, however, is not apparent on the 11th day following infection.

Autopsy findings: Seven days following infection of A-W and C-57 mice.—Cysts containing 1-12 small reddish worms were found in the muscular coat of the small intestine just under the serosa; these were most numerous in the first 2 inches of intestine but were also scattered posteriorly, in a few instances within $\frac{1}{2}$ inch of the ileocecal valve. No other pathological changes evident. Some worms were found migrating back into the intestinal lumen; empty cysts were seen filled with blood or as white discs against the pink intestinal wall.

Eleven days following infection of A-W and C-57 mice.—The abdominal cavity was almost normal. The intestinal wall in some cases was soft and glandular

in appearance with small blood-filled cysts; the duodenal wall often showed hemorrhagic spots at points of entrance of worms into the lumen. The mucosa occasionally had a "frosty" appearance, which was more pronounced later in the infection period. "Frostiness" was due to the color of the villi and was most evident in areas of worm concentration. Some animals already exhibited splenomegaly with some paling of the normal red color of spleen and also of the liver and kidneys. Symptoms were more pronounced in A-W strain.

Eighteen to 32 days following infection of C-57 mice.—The animals autopsied on the 18th day showed splenomegaly, with the spleen pale and rather cloudy in appearance; liver and kidneys were normal. The duodenum was enlarged, especially anteriorly, and glandular from gross exterior view; internally the mucosa was petechiated, frosty in appearance at the site of worm concentrations with some erosion of villi in duodenum; duodenal contents were normal except for excessive amount of liquid. On 25th day the spleen was normal or but slightly enlarged, but often dark with some cloudiness which imparted a bluish cast. Duodenum was similar in appearance to that on the 18th day.

On 32nd day the findings were the same as on the 25th day.

A-W mice dying after infection.—Those dying from infections of 500 larvae given at 32 days of age usually had symptoms and post-mortem findings similar to those with a dosage of 100 larvae. Death was sudden after onset of symptoms or sometimes without symptoms. Mortality started and ended earlier, 8–23 days following infection, and the pathological changes in the intestine were more pronounced than in mice given 100 larvae.

There was little or no evidence of diarrhea; intestinal trauma and hemorrhage was extensive; cysts on intestinal wall had few or no worms; empty cysts appeared as white to greyish discs, sometimes filled with blood and bright red; the spleen was not always enlarged, especially in those dying on 9th–12th days; in those dying later the spleen was sometimes twice normal size and light pink in color, and usually cloudy in appearance.

Infection Experiments in 5-month-old Mice

A-W and C-57 mice 5 months of age were infected with 500 larvae each of *Nematospiroides dubius*. Autopsies were conducted at 7, 11, 18, 25, and 32 days respectively following infection, using the same procedure as in the earlier experiments with 32-day old A-W and C-57 mice.

According to the experimental procedure here followed, it should be possible to compare 32-day and 5-month mice of both A-W and C-57 strains for a measure of age resistance. Two criteria are available as measures of resistance: (1) total worm recovery, and (2) rate of passage through the cyst phase in the worm life cycle. As a criterion of the latter the per cent of the total population present in the lumen of the intestine and the per cent still retained in cysts on the intestinal wall were determined for the 7th and 11th days respectively after infection. The latter criterion was chosen because of its ease of determination in the life history of this nematode.

Table 4 shows the average number of worms recovered from the two strains of mice at the several autopsy periods following infection. There is a significant difference between the number of worms recovered in the two strains, more worms having been recovered from the C-57 strain.

In Table 5 the worm populations of the animals in Table 4 for the 7th and 11th days have been broken down to show the distribution of their occurrence within the host. Five per cent of the worm population has reached the lumen by the 7th day in the A-W strain as compared to 64% in the C-57 strain and 94% on the 11th day as compared to 100% in the C-57 strain. The worms in the A-W strain are proceeding through their encysted stage more slowly than are those in the C-57 strain.

TABLE 4.—Total worm recovery averages with probable error of mean; dosage was 500 larvae per mouse; mice 5 months old, 5 mice per period

Days after infection	7	11	18	25	32
A-W strain, 2 died out of 26	411 ± 3.6	409 ± 6.6	416 ± 9.1*	427 ± 5.8*	356 ± 9.4*
C-57 strain, no deaths	459 ± 2.1	469 ± 4.8			

* Autopsies were not conducted upon the C-57 strain for the last 3 periods.

TABLE 5.—Average worm distribution from Table 4

Days after infection	7		11	
Worm recovery percentages	From cysts	From lumen	From cysts	From lumen
A-W strain	95 ± 0.5	5 ± 0.5	6 ± 1.7*	94 ± 1.7
C-57 strain	36 ± 0.6	64 ± 0.6		100

* Less than 0.1.

ACQUIRED RESISTANCE TO SINGLE SUPERINFECTION

To test the effect of a residual single initial infection upon a subsequent infection, 27 A-W mice were used in 3 groups (Table 6). Group A (6 mice) 32 days of age, was infected with 50 larvae per animal and autopsied at the age of 79 days. Group B (6 mice) 64 days of age, was infected with 100 larvae per mouse and autopsied at 79 days of age. Group C (15 mice) 32 days of age, was infected with 50 larvae each and again at 64 days of age with 100 larvae each, and the mice were autopsied at 79 days of age.

Groups A and B served as controls for the test animals of Group C, since Group A received only the initial infection, Group B only the second infection, and Group C had been given both the initial and subsequent infections. Therefore, if one initial infection of 50 larvae has any effect upon the number of worms recovered from the second infection, the average recovery from Group C should be less than that from Group A plus Group B.

The average worm recovery for each group in the A-W strain is given in Table 6. That of Group A was 41 worms, of Group B 96 worms (A and B combined, 41 + 96 = 137), and of Group C was 134 worms. Obviously, the two values, 134 and 137, are not significantly different.

The foregoing experiment was duplicated using C-57 instead of A-W mice. The results are given in Table 7. The average recovery was: Group A, 47 worms; Group B, 96 worms (A and B combined, 47 + 96 = 143), and Group C, 138 worms. Again there was no significant difference between test and control animals.

Length measurements were made of worms from each group of mice in the two experiments just described. Only males were measured because the female worms are coiled too tightly to make such measurements feasible. Length, as used here,

is the distance from the anterior end of the body to the anterior margin of the bursa. In the A-W strain, ten male worms were measured from each mouse of Groups A and B and ten worms from each of the first 6 mice in Group C. The lengths of the worms from Groups A and B were averaged to avoid possible effects of age differences in the worms. The worms of Groups A and B averaged 5.18 mm, and of Group C, 4.84 mm. A chi square comparison of these figures gives a value of 0.0111, not significant.

Such measurements were repeated for worms from the C-57 mice. Those of Groups A and B, averaged together, were 5.14 mm, of Group C, 5.10 mm. Again the difference has no significance ($X^2=0.0002$). It can be concluded, therefore, from the number and length of worms recovered, that no resistance was acquired from one residual primary infection of 50 worms in either strain of mice.

TABLE 6.—*Lack of acquired resistance to single superinfection—A-W strain*

	Dosage at 32 days of age	Dosage at 64 days of age	No. of worms recovered at 79 days of age
Group A, 6 mice	50	0	41 ± 1.5
Group B, 6 mice	0	100	96 ± 0.5
Group C, 15 mice	50	100	134 ± 1.2

$$A + B = 137$$

TABLE 7.—*Lack of acquired resistance to single superinfection—C-57 strain*

	Dosage at 32 days of age	Dosage at 64 days of age	No. of worms recovered at 79 days of age
Group A, 5 mice	50	0	47 ± 0.4
Group B, 5 mice	0	100	96 ± 1.1
Group C, 10 mice	50	100	138 ± 0.8

$$A + B = 143$$

At autopsy, Group A of each strain showed little pathological change, the only abnormality being a slightly "frosty" appearance of villi in areas of worm concentration. Group B of each strain showed some splenic enlargement, lightly frosted duodenal mucosa with petechiation, slight erosion of villi. Group C showed dull colored and slightly enlarged spleen; the duodenum was rather rough and glandular in appearance from exterior view; empty cysts on outside of the intestine were more distinct than in Group B, in some cases containing white to yellowish, caseous material.

DISCUSSION

It is clear from the foregoing experiments that the A-W strain of mice was very susceptible to a dosage of 100 larvae of *Nematospiroides dubius*. It exhibited a 26 per cent mortality when infected at that dosage while the other strains showed no mortality (Table 1). On the other hand, with a dosage of 500 larvae the mortality was 100 per cent in the A-W strain and started and ended earlier in the infection period.

In addition to the difference in mortality between the C-57 and A-W strains the worms passed through the encysted phase faster in C-57 mice 32 days old than in A-W mice the same age. In 5-month-old mice of the two strains this differential in rate of cyst passage was more pronounced.

When old and young mice were compared in the interval autopsy experiments the total recovery in 5-month-old A-W mice was somewhat less than in 32-day-old

mice of the same strain (Tables 2 and 3). In the C-57 strain there was no significant difference in total recovery between the two age groups.

A significantly smaller percentage of the total worm population was able to reach the lumen of the intestine by the 7th day in the 5-month-old A-W mice as compared to the 32-day-old mice of the same strain (Tables 3 and 5). With the C-57 strain the reverse was true, a larger percentage reaching the lumen on the 7th day in old as compared with young mice.

Unfortunately no experiments were carried out based upon a standard worm dosage per gram of body weight so that some of the differences between the two age groups may be due to differences in size or weight. However, not all the differences are ascribed to this factor since the rate of completion of the encysted stage was different in the two strains, having been slowed down in the A-W mice and speeded up in the C-57 strain with increasing age.

The A-W strain is susceptible to the infection on the basis of mortality as compared to the C-57 strain. However, since the parasite passes through its encysted stage slowly in the A-W strain as compared to the C-57 strain, the A-W strain might be considered resistant on this criterion. It is possible that the A-W strain is susceptible to the effects of the parasite but shows an actual resistance to the parasite itself. With the C-57 strain the reverse could be true, i.e., resistant to the effects but susceptible to the parasite. Thus it could be a more favorable host than the A-W strain. The fact that the C-57 strain allows the cyst phase of the life cycle to be completed more quickly in 5-month-old animals as compared to 32-day-old mice seems to point to its becoming a more favorable host with increasing age. This latter phenomenon then, may possibly be an inverse age resistance.

The differences noted between the strains of mice studied here seem most clearly explainable on a genetic basis since the different strains used were highly inbred and hence highly homozygous.

Among other workers, Graham and Porter (1934), Ackert (1935), Cameron (1935), Sheldon (1937), and Stewart, Miller, and Douglas (1937) have reported what may be genetic differences in animal hosts in relation to their parasites.

Little can be said of acquired resistance from the experiments reported here. Eliciting or detecting an acquired response might necessitate a larger initial dosage, several dosages, or other criteria of resistance.

SUMMARY

By infecting 3 highly inbred strains of laboratory mice with a standard dosage of *Nematospiroides dubius* larvae the writer has shown that the A-W strain is highly susceptible to the infection on the basis of mortality.

The C-57 strain is resistant on this basis but is a favorable host using as a criterion the rate at which the parasite passes through its encysted stage in the intestine of the mouse.

The C-57 strain on the latter criterion is a more favorable host at 5 months than at 32 days of age while the reverse is true of the A-W strain.

On the basis of total worm recovery the A-W strain is slightly more resistant at 5 months than at 32 days of age although part of the age differences in these strains may be due to increase in weight of the mice.

The strain differences demonstrated here appear to be most easily explainable on a genetic basis.

No conclusions are drawn regarding acquired resistance except that it was not observed under the conditions of this experiment.

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A CONTRIBUTION TO THE KNOWLEDGE OF THE RODENT WARBLE FLIES (CUTEREBRIDAE)

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The rodent warble flies are myiasis-producing DIPTERA restricted to North and South America, the larvae of which inhabit the subcutaneous tissues mainly of rodents, lagomorphs, and small domestic animals. The adult flies, which are rarely observed in nature, have reduced mouthparts and do not feed.

References to the habits or biology of these flies are scarce, even though larvae are frequently encountered by veterinarians and others, especially in relation to infections of cats and dogs.

The author has been informed by veterinarians from various parts of the United States that these larvae are commonly found in cats and dogs. Personal reports from midwestern states, particularly from Ohio, indicate that it is common practice for owners to remove larvae from beneath the skin of pets every summer, and the Small Animal Clinic, New York State Veterinary College, Ithaca, N. Y., is frequently called upon to remove such larvae. These cases are sufficiently common that Cornell veterinarians approximate the infection period as early July to late September, with a peak from mid- to late August. At the New York State Fair at Syracuse in 1939, many people observing the exhibit of parasites of domestic animals, were overheard saying that their cats and dogs often get such infection during the summer.

Many workers have conjectured that infection of cats and dogs is solely accidental and occurs only when these animals eat infected rodents and lagomorphs. However, nursing kittens are often found infected. Dr. H. H. Schwardt (Cornell University), and others, have found their house cats, which never roam through the fields, infected with *Cuterebra horripilum* Clark, a species usually infecting cottontail

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rabbits. Such findings, as well as many *Cuterebra* infections of cats and dogs seen by the author, indicate that these animals may commonly serve as hosts of *Cuterebra* species.

A few cases of *Cuterebra* infection have been recorded from opossums, hogs, and asses, and one case from man. The author has one mature larva of *Cuterebra horripilum* removed from the trachea of a pig at the New York State Veterinary College in August, 1942. These larger animals, however, are not frequently found infected with cuterebrae, and the unusual points of localization of the larvae in these hosts indicate that such infections are accidental.

Besides such circumstantial evidence, much experimental field and laboratory work must be done before we know accurately the mode of infection of these parasites, their path, if any, through the body, and the effect upon the host.

OBSERVATIONS

The following observations of *Cuterebra* infections were made by the author during 1940 and 1941 at Ames, Iowa. Extensive trapping was carried on of the north-

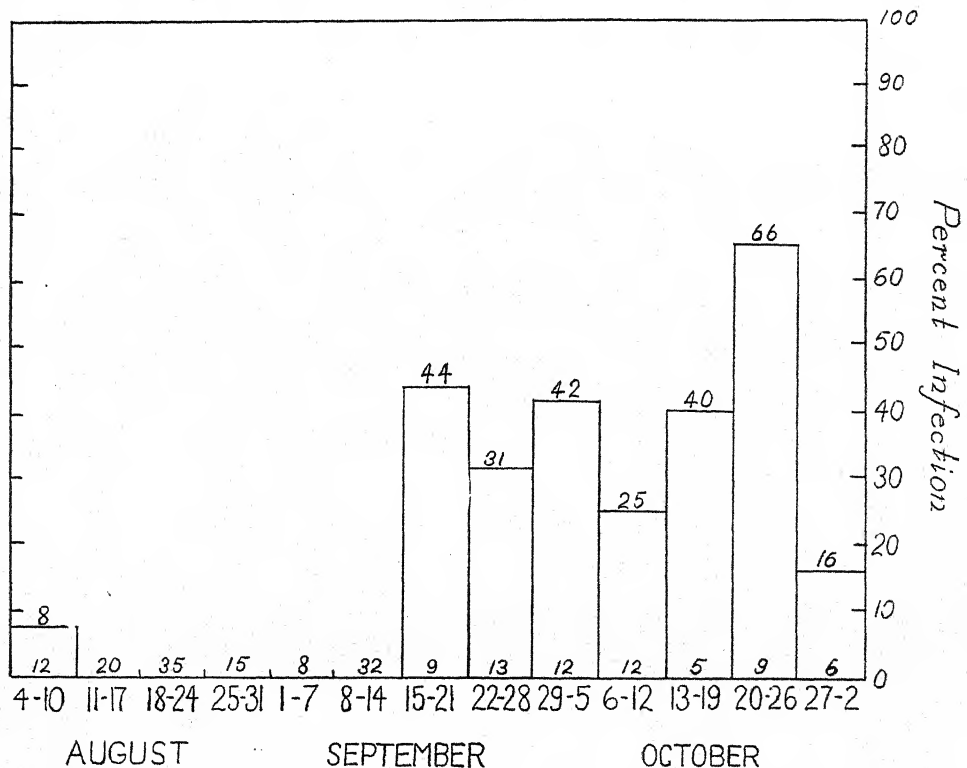


FIG. 1. Seasonal infection of northern white-footed mouse with *Cuterebra peromysci* during 1940. Numbers at bases of columns represent the numbers of mice caught for the corresponding week periods.

ern white-footed mouse (*Peromyscus leucopus noveboracensis*) which was found to act as the host of the larvae of *Cuterebra peromysci* Dalmat (1942). Traps were set daily from early summer to mid-winter during 1940. The infection period extended from September 15 to November 2, during which time 25 of the 68 mice caught (38

per cent) were parasitized. Excluding the one parasitized mouse trapped August 4, infection was not encountered at any other time during the trapping period (Fig. 1). It will be noted that more mice were caught during the period in which no infection was found.

In 1941, 54 per cent of the mice trapped in the same area were found to harbor *Cuterebra* larvae. The larvae were located predominantly in the general inguinal region, each larva with its posterior spiracular plates exposed through a hole in the host's skin. Several mice harbored between three and five of them and one mouse bore seven in different stages of development.² (One of these larvae was a first instar.) Young mice were seldom found parasitized. Almost all infected animals were awkward, many of them attempting to remove the bulging larvae with their claws and teeth. When a male mouse harbored more than one parasite, total emasculation usually resulted. About 20 per cent of the infected mice were found dead in their cages at about the time the larvae started to emerge.

Although infection by these parasites is often high, it is local in distribution. Almost all the infected mice were caught by the author in an area about four acres in size, while similar types of areas about 500 yards away with numerous mice yielded no infection. This has also been the experience of others who encounter *Cuterebra* in field animals but is not surprising as most species of warble flies, although common, are localized in comparatively small areas.

Trapped mice were brought into the laboratory, examined for warbles, and then placed in hardware-cloth cages kept over receptacles containing slightly moistened earth. When the study was begun, the *Cuterebra* larvae were permitted to emerge naturally, but after it was discovered that the mice sometimes attacked, killed, and ate the maggots while they were emerging, the maggots were extracted from the mice just as they started to leave. This was accomplished by gently squeezing the skin of the mouse from behind the inner (anterior) end of the maggot. After a minute or two the maggot would loosen its hold and fall out. The larval "cyst" was not walled like that of the ox warbles, but was just an area in which necrosis of the muscle tissue had occurred. A scab usually formed in from one to two weeks, although in some cases the wound persisted for a longer period, continuously discharging pus and serum. All mice were retained for proposed infection experiments. A thorough internal examination of 50 infected mice was made in an attempt to discover a possible path of larval migration in the host. However, larvae in all stages of development were found only under the skin.

When the larvae left the host they were placed on earth into which they burrowed almost immediately, and within two days puparia were formed at a depth of one and

² This high per cent of infection and the large number of parasites per host are not rare occurrences. According to Vorhies and Taylor (1940) *Cuterebra* species are the most conspicuous parasites of *Neotoma*. In 39 consecutive examinations of these rats made by Spencer in the Verde Valley, 24 were found to be infected (almost 62 per cent), and as many as four or five larvae were found in some rats. Females were found with nursing young, all three being infected. Gander (1929) found about 50 per cent of the woodrats he examined parasitized and Merriam (1889) found about 50 per cent of the chipmunks at the south end of Lake Champlain infected with these parasites. Bailey (Vorhies and Taylor) recorded that each of five woodrats taken near Cliff, New Mexico had from one to three in its throat while Cameron (1926) reports that a rat harbored 17 larvae. Mr. Everett W. Jameson, Jr., informed the author that during a few weeks of trapping at Point Abino, Ontario, in the summer of 1942, he found almost every deer mouse to be the host of one to four warble fly larvae which came to rest alongside the testes in the males. He found the mouse population in his trapping area very low in comparison with previous years.

one-half to five inches. It was noted that the puparia (with the enclosed pupae) were only about four-fifths as heavy and four-fifths as long as were the larvae before pupation. This must have resulted primarily from a loss of moisture and is probably an adaptation to prevent excessive freezing of the overwintering pupae. The above measurements were made from the records of thirteen larvae and the resulting puparia.

After measurements were taken, the puparia were placed in two pails of earth which were kept in an outdoor subterranean basement during the winter months.

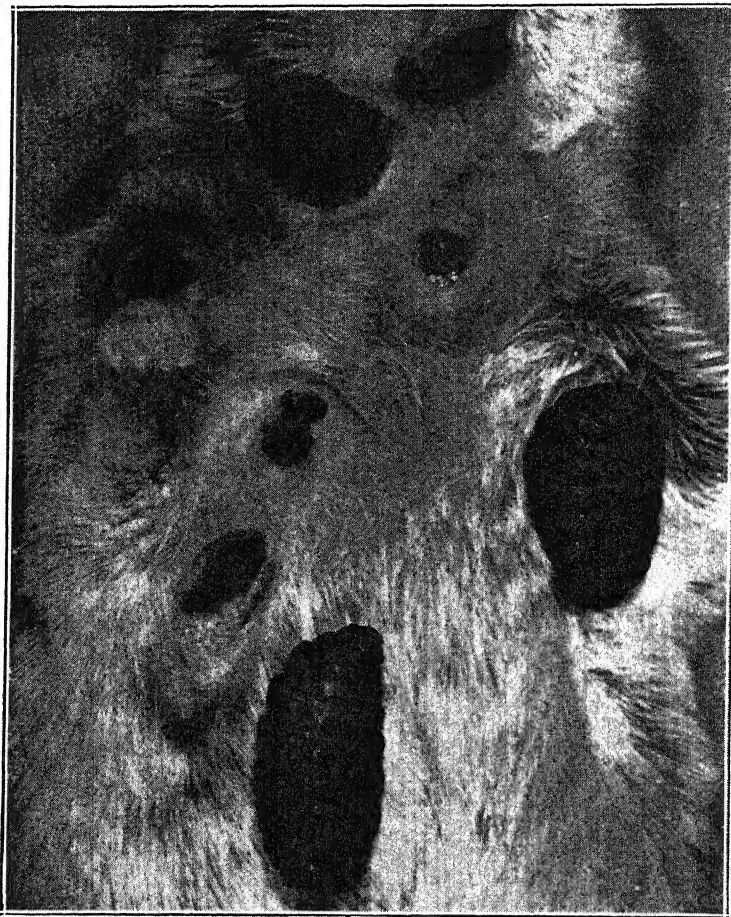


FIG. 2. *Cuterebra* larvae in various stages of emergence from rodent host. Note that the posterior end leaves first. (Photograph by George McClellan Bradt.)

Thermograph records showed that the temperature of the soil in the pails varied from 25 to 41° F.

In mid-April, 1941, the pails were removed from the basement and kept outdoors. An outdoor cage, 7 × 5 × 5 feet, constructed of window screening on a wooden frame and without a bottom, was built to house the flies when they emerged. Another cage, 4 × 4 × 2½ feet, constructed of sheet metal and with an open top, was filled with a layer of earth and nesting material and was stocked with ten mice. This cage, along

with the pails containing the pupae, was placed in the larger cage, the latter being partially covered with cheesecloth to simulate the mottled shade and sun of the woodlands. It was hoped that the emerging flies would have ample room to react normally in the presence of their host and that their method of oviposition would thereby be learned.

The first adult fly, a female, emerged May 31. When the fly was disturbed it made a loud buzzing noise, louder than, though distinctly different from that of a bumblebee. On June 1 a second fly, a male, emerged. The flies were quiet and it was usually difficult to find them since they remained well-hidden within a hollow log or on the underside of leaves. When the leaf upon which a fly was resting was picked up, the fly still did not move. Although they were clumsy on their feet, they were excellent flyers. At no time did the flies seem attracted to the mice, but they often were seen near the entrance of the mouse burrows. The male fly died in three days and the female disappeared after ten days, oviposition not having been observed.

Since so few flies had emerged, the remaining puparia were dug up and four of them were found to be empty. It is likely that the flies that had emerged from these puparia, as well as the one missing fly mentioned above, landed near the mice and were eaten. On August 21 another fly, a male, emerged and was immediately pinned.

In 1942 the author obtained a number of adult *Cuterebra peromysci* which emerged from puparia collected the previous year. The eggs removed from one dissected female, as well as those laid on twigs and paper by another, averaged 1.4 mm in length and 0.3 mm at the widest point. They displayed a very heavy chorion, a ventral groove, a sticky covering on the caudal end, and an operculum on the dorsal aspect of the cephalic end. Approximately 450 eggs were removed from the dissected female and the living fly deposited a like number. Sporadic egg-laying began two days after the emergence of the female, the fly ejecting one egg after another for a time, then moving about a bit and depositing no eggs, then laying one or two eggs, and occasionally refraining for long periods. About 45 hours were required for the deposition of all the eggs. The eggs never completely overlapped each other although several shingled masses were formed. When ovipositing on a twig, the female encircled it with her eggs.

DISCUSSION

The method by which *Cuterebra* larvae gain entrance into the host has been open to much conjecture. Some have assumed that the ventral groove of the egg indicates that the eggs definitely are deposited on the hair of the host, and that the operculum can only be opened by friction and moisture applied by the tongue of the host when licking its fur. It follows that the larvae which are taken into the mouth penetrate the tissues, migrate to the subcutis where they mature, and finally emerge through holes they produce in the skin of the host. Others modify this approach. They believe the eggs are laid on food plants of the host and that when the latter feeds, the eggs are also ingested. Friction and moisture in the mouth again cause the operculum to open and the rest of the development is the same as has already been described.

Moilliet (1943), and also Ferris (1920), however, have had the larvae emerge from eggs with no mechanical aid. Should the eggs be deposited on the host, the

tremendous numbers laid by *Cuterebra* species (more than 1000 eggs have been taken from one *Cuterebra horripilum*) would be unnecessary. Since the large number of eggs produced indicates that oviposition does not occur on the host, the first instar larvae must have a reduced chance of reaching a suitable host, and therefore a high mortality rate. Parker and Wells (1919), and Moilliet found eggs to remain viable for at least six months and Moilliet kept one unfed larva alive for ten days. Both of these investigators found the newly emerged larvae to be very active. These specializations seem unnecessary if the female deposits her eggs directly on the host animal.

Infection through the mouth precludes the possibility of infection of those animals that have not yet begun to feed. However, Mr. Ralph H. Smith, Game Research Investigator, New York Wildlife Research Center, found a six-day-old cottontail already parasitized (personal correspondence) and Spencer (Vorhies and Taylor, 1940), found female woodrats and their nursing young all infected.

It is not surprising to find that some of Parker and Wells' transfers of larvae to the mouths of prairie dogs were successful since there is no reason to suppose that larvae, that do happen to reach the mouth cavity, cannot penetrate the mouth tissue. However, it will be noted that over three-fourths of the larvae failed to become implanted and that some larvae were found dead in the host tissue. This might be due, in part, to the difficult migration the larvae would have to make before reaching their ultimate points of localization under the skin of the host.

The very heavy chorion of the *Cuterebra* eggs indicates that they are laid intact and that the enclosed larvae do not emerge for some time after oviposition. As indicated in the author's observations of adult *Cuterebra peromysci*, the flies of this group seem to be stealthy and usually never come in contact with the host. Townsend (1935) observed many of these flies in the wild and has seen them fly to rock-piles, small recesses in rocks, and wood or brushpiles that might have formed rodent habitations.

It seems probable that the eggs of *Cuterebra* species are deposited among the nests, burrows, or logs used by the hosts. The young larvae emerge intermittently, become attached at their posterior end, and sway up and back while awaiting a suitable host. When they come in contact with such an animal, they attach to it, perhaps move about over it, and burrow in when a satisfactory spot is reached. They continue their development in the subcutis of the host. Since definite cyst walls are not formed around the larvae, it is possible that the larvae may migrate a distance under the skin while feeding. They finally emerge through either the hole they formed when entering, or, if they migrated, through another hole produced elsewhere. Although this relationship of larva to host is supported by much circumstantial evidence, it cannot be proved definitely until additional observations have been made.

Another problem arising in the study of the cuterebrid parasites is the length of larval development in the host. Both Vorhies and Taylor, and Moilliet concur on this point, finding the larval period of *Cuterebra tenebrosa* Coq. to be from about three weeks to a little over a month. Scott and Snead (1942) were able to determine the infection period of a mouse-infecting species (probably *C. peromysci*) as approximately one month.

The pupal period of *C. tenebrosa* under laboratory conditions was found by Parker and Wells to be 47 days in one instance and 173 in another, while Moilliet found it to be about 11½ months for a pupa of the same species kept in an outdoor insectary. The pupal period of *C. peromysci*, the larvae of which were taken during the period from September to November by the author, was from 8 to 10½ months, while it was less than two and one-half months for one pupa, the larva of which was taken in mid-July. The large range of pupal period indicated in the records of Parker and Wells, and of the author, may be accounted for in part by the amount of food stored in the puparia, the moisture, temperature, and other environmental factors. Another consideration might be the time of year in which pupation occurred. The pupal stage may be short during mid-summer and very long if initiated during the fall and winter.

It is generally agreed that in North America *Cuterebra* species overwinter in the pupal stage. However, the heavy chorion of the eggs, the long period of incubation before larvae begin to emerge from the eggs (Moilliet found this period to be over a month under laboratory conditions), the ability of the eggs to remain viable for at least six months (Parker and Wells, and Moilliet) and to withstand sub-freezing temperatures (Moilliet) indicate the possibility that at least some species may also overwinter as eggs. It is likewise assumed that there is only one generation per year. However, the short pupal periods of two different species reared by Parker and Wells, and the author indicate that there might be an additional generation during the summer months. In both cases the flies developed from larvae that were taken early in the summer.

Lastly, the effects of these parasites on their hosts should be mentioned. Although in a few of the reported cases of *Cuterebra* infection the larvae emerged without having inflicted any apparent hardship on the host, most investigators have observed that infected rodents were awkward and therefore easy prey for predators. Scott and Snead found a correlation between the decrease in mouse populations and the peak of *Cuterebra* infection. Jameson, in his trapping of deer mice at Point Abino, Ontario, found the mouse populations greatly reduced in relation to what they had been in previous years but the mice he did find were almost all parasitized. Lindquist (1937) has shown that the warble, *Cuterebra buccata* Fab., was the principal predisposing cause of infection in cottontail rabbits in Uvalde, Texas, by the primary screwworm, *Cochliomyia americana* C. and P.

Dr. Kenneth Bogard (personal communication) removed a larva of *Cuterebra horripilum* from the neck of an eight-week old kitten and also one from a litter-mate. Both kittens were thin and unthrifty. Crushing of a *Cuterebra* larva in an attempt to remove it from under the skin of a dog caused symptoms like those of anaphylaxis (Cameron). Rabbit breeders have mentioned to the author that it was often necessary for them to kill some of their stock because of the large size of the wounds caused by these larvae. Moilliet found that infection with these parasites led to the death of many of his experimental rats and Parker and Wells found one of their three experimental prairie dogs seriously affected by a larva. The author had about 20 per cent of his infected mice die from unknown causes just at the time the larvae were emerging (this does not include those that died of *Salmonella* poisoning). It has been the experience of Cornell veterinarians that wounds in cats and dogs

caused by these larvae are dirtier and of a longer duration than those caused by other myiasis-producing DIPTERA. This might be accounted for by the lack of a definite cyst wall surrounding the larva, which condition would permit more easy infiltration of toxic materials into the surrounding tissues. The wounds do not start to heal for a long time, often remaining open for weeks with an accompanying discharge. Pyemia, with resulting death, sometimes occurs.

Cuterebra larvae also affect the food value of animals. Mr. A. C. Shaw, Assistant Regional Forester, Atlanta, Georgia, notes: "Infestations in the larval state have been noticed in this region on squirrels during the early fall hunting season. Natives refer to these as warbles, and usually infested squirrels are discarded as inedible" (personal communication).

These parasites do have a definite affect on rodent populations and must therefore be a factor in the control of rodent ectoparasites that act as vectors of important human and animal diseases. It would appear, therefore, that this group of myiasis-producing flies deserves greater attention than it has received in the past.

SUMMARY

Original and recorded observations in *Cuterebra* species are discussed from the following standpoints: (1) The method by which *Cuterebra* larvae gain entrance into their hosts, (2) oviposition, (3) length of larval development in the host, (4) length of the pupal period, (5) overwintering of the parasites, and (6) the effect of the larvae on their hosts.

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IN VITRO CULTIVATION OF TREMATODE METACERCARIAE FREE FROM MICROÖRGANISMS

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Cercariae of the strigeid trematode *Diplostomum flexicaudum* (Cort and Brooks, 1928) naturally develop into metacercariae in the eye lenses of many species of fishes (Ferguson and Hayford, 1941). Recent studies have shown that the metacercariae of this fluke will also grow normally in the lenses of frogs, turtles, chicks, ducklings, and laboratory mice, rats, guinea pigs and rabbits (Ferguson, 1942). This is a report of experiments in which it has been found that axenic* cercariae of *D. flexicaudum* will develop at room temperature into metacercariae when placed in culture media containing lens material from the eyes of vertebrates representing both cold- and warm-blooded groups.

MATERIALS AND METHODS

Cercariae used in these experiments came from laboratory-reared snails (*Lymnaea stagnalis*) infected with a strain of *D. flexicaudum* obtained through the courtesy of Dr. F. G. Wallace, University of Minnesota. The larval worms were allowed to emerge in half pint milk bottles containing a little aquarium water. For use in certain experiments they were concentrated to form a heavy suspension in water by slow speed centrifugation in special tubes elsewhere described (Ferguson, 1943).

The cercariae had to be free from contamination with microörganisms before they would survive and develop in cultures. This is in accord with observations made by various authors who have attempted to obtain development in vitro of parasitic worms of vertebrates (Ferguson, 1940). It has not been possible to obtain axenic cercariae for inoculation into cultures by washing the larval worms in sterile water, nor did they survive treatment with any one of several chemical sterilizing agents used at various dilutions. The cercariae were, however, freed from contamination as they penetrated a susceptible fish host and migrated to the eye for localization in the lens. Hundreds of larval worms were found in the lenses of blackhead minnows (*Pimephales promelas*) and fingerling rainbow trout (*Salmo irideus*) if a few of these fish were allowed to swim for about an hour in a small amount of water containing several thousand recently emerged cercariae. Fish so treated showed a heavy mortality, but this could be avoided if the organisms were concentrated as described above and were injected into the orbital space by means of a syringe needle inserted beneath the tissues surrounding the eyes. From this location large numbers of cercariae migrated into the eye and reached the lens within a few hours. This

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* The adjective *axenic* was introduced to denote a living organism that is free from all other demonstrable organisms (Baker and Ferguson, 1942, Proc. Soc. Exper. Biol. and Med. 51: 116). It is a more convenient term than expressions currently used, e.g., "animals free from microörganisms," "animals free from contaminating organisms," and "animals free from contaminants" and in meaning it is more correct than the adjectives "sterile" and "germ-free." Axenic organisms are defined as individuals of a species free from any life apart from that produced by their own protoplasm.

method of obtaining large numbers of cercariae in the lenses has also been used with *Lepomis macrochirus*, the bluegill sunfish, which acquired few or no larval worms even when exposed over several hours to thousands of cercariae in a small amount of water. A few dozen cercariae eventually reached the lenses of bluegills when large numbers were injected into the coelomic cavity.

In some experiments cercariae were allowed to reach the lenses of the above-mentioned fish, and then the lenses were removed aseptically and introduced directly into culture media where development occurred. In other experiments where uninfected fish lenses formed part of the culture medium, and in all experiments in which lens material from animals other than fishes was used, the axenic cercariae were first recovered from the lenses into which they had migrated. This recovery was accomplished as follows. A few hours after exposure to cercariae, the fish were killed and the lenses removed aseptically with sterile forceps through a cut in the cornea from eyes which had previously been painted with tincture of iodine and then flamed lightly with a gas burner. Several lenses were dropped into a tube containing Tyrode solution. Using bacteriological precautions the lenses were macerated in the test tube with a glass rod. This procedure liberated most of the cercariae. The lens material and cercariae were transferred in a pipette to a sterile centrifuge tube of Tyrode solution, where the larval worms were allowed to filter through stainless steel wire cloth (80×80 mesh per inch), thus separating themselves from most of the lens debris. The cercariae settled through the salt solution into the tapered end of the centrifuge tube where they were recovered in a pipette for inoculation into culture tubes.

By carefully following these procedures axenic cercariae were regularly obtained, and no microorganisms could be demonstrated when samples of these larval worms were inoculated into routine laboratory media under aerobic and anaerobic conditions.

All cultures were contained in small test tubes (13×100 mm). These tubes were stoppered with short cotton plugs over the surface of which a layer of sealing wax was applied in order to prevent evaporation during the several weeks required for the development of the metacercariae. All culture experiments were carried out at room temperature.

MEDIA AND GROWTH IN STERILE CULTURES

In early experiments recently infected lenses of bluegill sunfish that had been exposed to cercariae by one of the methods described above were added individually to the small test tubes containing varying amounts of one of the following liquid media: Tyrode solution, Tyrode solution plus chicken serum, Tyrode solution plus a special bakers' yeast extract, bovine aqueous humor, and bovine vitreous humor. A few tubes contained Tyrode solution that had been injected into the coelomic cavity of large bluegill sunfish and withdrawn after two hours. Similar methods were used by Weekers (1939) for obtaining a medium in which rabbit lenses would survive and remain transparent and intact.

Varying degrees of development of cercariae toward the metacercarial stage occurred in these different cultures with the exception of those containing either bovine aqueous or vitreous humor. In this connection it has been found that these larval trematodes will penetrate the eyes of blackhead minnows and young rainbow trout from which the lenses have been removed previously, but they survive for only about a day in the humors of the eye. In many of the cultures the lenses remained intact

and transparent for 2 or 3 weeks and the developing metacercariae could be observed in the culture tubes when the latter were held horizontally under a dissecting microscope.

Cultures containing only the infected lens and either one or two cubic centimeters of Tyrode solution yielded fully mature metacercariae after about 3 to 4 weeks. When axenic cercariae were added to cultures containing an uninfected sunfish lens the larval worms were able to penetrate the lens and develop into metacercariae. Every culture experiment did not yield metacercariae. In many tubes the cercariae would show a small amount of development and then die. Whether there were significant differences in the lenses is not known. However, it was found that cercariae never survived long or showed much development when infected rainbow trout lenses instead of those from bluegills were placed in cultures. Metacercariae developed in lenses that remained intact and transparent or became opaque while remaining intact, and also in tubes where the lenses had disintegrated within a few days to form a flocculent mass.

As many as 155 and in many cases between 75 and 100 metacercariae developed in a culture containing but a single sunfish lens. Where the number of larval worms in a culture was this large, they tended to vary in size from very small partially developed forms to fully mature and large metacercariae, even after the cultures had been held for over 7 weeks. When fewer metacercariae were present in a culture they were usually larger. Metacercariae developing in this medium have lived and remained fairly active in cultures that were held over 4 and 6 months respectively. Usually, however, the metacercariae began to die or became inactive and opaque two months or more after the cultures were initiated. This is in contrast to the survival of metacercariae for over 15 months, and perhaps years, in the lenses of fishes, the natural hosts.

Fully developed metacercariae have been obtained in cultures consisting of two cubic centimeters of Tyrode solution and the lens of a laboratory rat. Here axenic cercariae were introduced into the tubes after the lens had been added to the salt solution. No survival or growth of axenic cercariae occurred in cultures where the rat lens was replaced by a small piece of either a rabbit lens or bovine lens (pieces the size of or a little larger than sunfish lenses, which are 3–4 mm in diameter). In contrast to these results many large and active metacercariae normal in appearance have been obtained in cultures containing rabbit or bovine lens substance but where the Tyrode solution was replaced by frog Ringers solution.

When the cultures were first initiated the pH of the medium was usually about 7.8. After several weeks when the metacercariae were mature the pH of the media varied from slightly below 7.0 to 7.5.

The infectivity of metacercariae developing in many of the culture experiments has been tested by feeding large numbers of these larval worms imbedded in the bodies of small rainbow trout to laboratory-reared laughing gulls (*Larus atricilla*) or by injecting them directly into the duodenum of 2 day unfed baby chicks. Adults of *D. flexicaudum* have not yet been obtained.

Data concerning the axenic metacercariae developing in cultures are given in Table 1. For comparison, data on metacercariae growing in the eyes of both cold- and warm-blooded vertebrates are also given. Of prime interest in the table is the range in body size of the fixed metacercariae from the various sources. A con-

siderable size variation exists among the metacercariae in each collection. The size of the larval worms developing in culture is in some instances greater than that of metacercariae recovered from the eyes of living animals.

TABLE 1.—Data, including range in body size, concerning metacercariae of *D. flexicaudum* developing in sterile cultures and naturally in the eyes of experimentally infected cold- and warm-blooded vertebrates

Source of metacercariae	Period of time larval worms were present in cultures or in vertebrate eye	Condition of metacercariae before fixation	Range in body size* of 10 metacercariae after fixation†	
			Length	Width
<i>Cultures</i>	<i>Days</i>		<i>mm</i>	<i>mm</i>
1 ml Tyrodes + one sunfish lens	52	Active, normal	0.27–0.60	0.09–0.30
2 ml Tyrodes + one sunfish lens	52	in appearance	0.27–0.57	0.09–0.25
2 ml Tyrodes + one rat lens	46	ditto		
		or opaque and dead	0.15–0.28	0.07–0.12
2 ml frog Ringers + piece of rabbit lens	46	Active, normal		
2 ml frog Ringers + piece of bovine lens	46	in appearance	0.27–0.63	0.09–0.27
		Active, but slightly opaque	0.22–0.49	0.09–0.16
<i>Eye of vertebrate</i>				
Bluegill sunfish (<i>L. macrochirus</i>) No. 1	70 +	Active, normal		
		in appearance	0.31–0.60	0.18–0.31
Bluegill sunfish (<i>L. macrochirus</i>) No. 2	60 +	ditto	0.31–0.39	0.13–0.19
Frog (<i>Rana pipiens</i>)	57	ditto	0.25–0.35	0.13–0.17
Laboratory mouse	26	ditto	0.45–0.50	0.16–0.20
Laboratory rat	27	ditto	0.20–0.40	0.10–0.17

* Range in size of body of 10 cercariae recently emerged from snail host and fixed in hot alcohol-formalin-acetic solution was 0.10–0.18 mm in length and 0.03–0.04 mm in width.

† Fixation in hot alcohol-formalin-acetic solution.

DISCUSSION

Axenic cercariae of *D. flexicaudum* remain alive in Tyrode solution, without lens material, for only about one week and little or no development occurs. It can be concluded from the experiments that the lens material from the various sources must contain the principal substances necessary for the growth of the cercariae into metacercariae. The fact that development occurred in cultures containing lens materials from fish, rats, rabbits, and cattle shows that the chemical composition of the lens materials from the different vertebrate species must be rather similar. These results were not surprising after it had been determined that the cercariae could survive and develop in the lenses of living laboratory mice, rats, guinea pigs, and rabbits (Ferguson, 1943).

Here it should be mentioned that the lens proteins of more or less closely related vertebrates within a class are apparently similar (Markin and Kyes, 1939), while differences have been demonstrated in lens proteins of animals belonging to separate classes (Ecker and Pillemer, 1940). These chemical differences are apparently not great enough to make the lens substance from any of the particular animals employed in my experiments unsuitable nourishment for the developing metacercariae.

It is significant that metacercariae developed in fish and rat lenses that disintegrated or became opaque soon after being introduced into the cultures and in tubes containing pieces of rabbit and bovine lens. If development occurred only in intact lenses in a culture medium where they remained transparent it would have to be admitted that the larval worms required an environment similar to that found in tissue cultures. These findings are encouraging when one contemplates the problems involved in analyzing the culture medium to determine the specific nutritive requirements of these larval helminths.

The salt content of the medium is an important factor and apparently requires variation depending on the source of the lens substance. This is clearly shown by the fact that metacercariae developed in Tyrode solution plus either sunfish or rat lenses but failed to grow in Tyrode solution plus lenses from rainbow trout or pieces of rabbit or bovine lens. However, development occurred in cultures containing pieces of rabbit or bovine lens when frog Ringers solution was used instead of Tyrode solution. The necessity of determining the proper salt content in the medium is emphasized by Stoll (1940) in his sterile culture experiments with the nematode *Haemonchus contortus*, and Ferguson (1940) in describing the cultivation of axenic adults of the trematode *Posthodiplostomum minimum*.

The presence of a great many small partially developed metacercariae in cultures where the larval worms were present in large numbers was to be expected. Their failure to grow larger was probably due to several factors among which the smaller amount of food material, an unfavorable oxygen balance, and the accumulation of excessive amounts of waste products were likely important.

SUMMARY

Methods are described for obtaining the cercariae of the strigeid trematode *Diplostomum flexicaudum* free from microorganisms. When introduced into culture media consisting of either Tyrode solution or frog Ringer solution and lens materials from fish, rats, rabbits, or cattle, these axenic cercariae developed into metacercariae that were morphologically normal, but they failed to infect chicks or laughing gulls.

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PRELIMINARY STUDIES ON THE PHYSIOLOGY OF *Aedes*
aegypti (DIPTERA: CULICIDAE). I. THE HATCHING
OF THE EGGS UNDER STERILE CONDITIONS

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The study of the metabolism of the egg and larval stages of mosquitoes requires their culture under sterile conditions. This has been accomplished in a number of cases by sterilization of the surface of the eggs and their subsequent introduction into sterile media, but the varied methods employed by previous workers have given inconsistent results regarding the influence of living micro-organisms on the hatching of *Aedes aegypti* eggs. This study was undertaken to settle the point.

Atkin and Bacot (1917) recorded that the presence of bacteria or their products stimulated the hatching of the eggs of this mosquito. This stimulation was demonstrated to be apart from the alkalinity or acidity produced by bacterial growth. Living cultures of *Staphylococcus aureus* and *Bacillus coli communis* caused the immediate hatching of dormant eggs. Eggs also hatched in the autoclaved extracts of these bacteria. The filtrate of *Bacillus coli communis* was not effective in inducing the eggs to hatch. Living cultures of yeast were similar to bacteria in effecting the hatching of the eggs. Though they ran no tests on molds, observations in cases of breakdown in sterility indicated that they had a similar effect.

Roubaud and Colas-Belcour (1927) observed that in pure or only slightly impure water the eggs of *Stegomyia fasciatus* (*Aedes aegypti* L.) may lie dormant for months, but hatch in water rich in organic matter, and containing bacteria and yeasts. They also found them to hatch in living cultures of *Bacillus coli* and yeasts, and in filtered extracts of such cultures. These authors believed that soluble ferments, which are contributed by bacteria, are responsible for the hatching of the eggs. They found that the enzymes, pepsin, papain, trypsin, and tyrosinase seemed to cause the eggs to hatch. They were unable to induce hatching with the digestive or fermentative products of proteins, i.e., urea, glycocholic acid, alanine, indol, etc. Likewise, the acids and alkalies of fermentation (lactic acid, ammonia, carbon dioxide) failed to cause the eggs to hatch. Later Roubaud (1927) reported that the action of calcium hypochlorite simulated the action of the proteolytic enzymes in stimulating the eggs to hatch.

Hinman's results (1930) disagreed with those of the above authors. He found that the eggs of this species hatched as readily in sterile as in contaminated media. Barber (1928) also reported no indication in his experiments of any promotion of hatching by bacterial growth.

MacGregor (1929) described the normal development of *Aedes aegypti* from egg to adult on a sterile medium consisting of bread in distilled water. Hinman (1932) and Rozeboom (1935) attempted to repeat MacGregor's work, but succeeded only in producing development of the larvae to the second instar. Trager (1935) found that medium consisting of 0.5% Eli Lilly Liver Extract No. 343 in

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combination with a 1% suspension of either brewer's or baker's yeast was sufficient for the hatching of the eggs and the normal development of the larvae under sterile conditions.

MATERIALS AND METHODS

A colony of mosquitoes was maintained to furnish eggs for the tests. These eggs were laid on a filter paper disc partially lining the sides of a beaker about half full of distilled water (method of Trager, personal communication). Except where otherwise stated, the eggs for each test were collected during a twelve-hour period and were kept moist for twelve additional hours before sterilization and introduction into culture tubes.

A modification of Trager's (1935) technique was used in sterilizing the surface of the eggs. "Bowls" were made of Will Corporation's 10 mm circular cover glasses by flaming the edges until they turned under to form an oval-shaped bowl. At the time of sterilization the desired number of eggs was forced down through the surface film of water contained in the bowl. White's solution, as employed by Trager (1937), was used for the sterilization of the egg surface, since a comparative test of the effectiveness of this agent had shown it to be far more effective for this purpose than either hexyl resorcinol, sersan, or merthiolate. The sterilizing agent was run into a sterile petri dish and the bowl of eggs was sunk into the solution. The few eggs which were driven from the bowl by convection currents were returned with a capillary pipette. The cover was placed on the petri dish and left for the duration of the twenty-minute period of treatment. Forceps, well flamed, were used to transfer the bowl of eggs to a pyrex culture tube (19 × 150 mm) containing 10 ml of sterile distilled water. One of the eggs was then taken with a sterile capillary pipette and introduced into each of the culture tubes which contained 5 ml of media. Nutrient broth, sterilized by autoclaving, was used as a medium except in cases otherwise specifically stated. The value of nutrient broth for this purpose lies in its becoming cloudy with bacterial growth, thus serving as an indication of contamination. The cultures were incubated at 26° C to 29° C, and observations of the cultures were made at regular intervals of twelve hours.

EXPERIMENTAL DATA

Evidence that contamination of medium stimulates hatching.—In one test, 100% sterility of the egg surfaces was effected by the treatment of eighty-four eggs with the germicide. However, only one of these eggs hatched by the end of the fifth day of incubation. Thirty-eight of the sterile cultures were then exposed to contamination by removing the cotton plugs. Twenty-six of these contaminated tubes showed hatching by the end of the eighth day, while no further hatching occurred in the 46 tubes kept sterile.

It seemed possible that this apparent lack of harmony with the results of Trager (1935) may have been due to the media used, since Trager had allowed the eggs to hatch in a 0.5% solution of liver extract (Eli Lilly, Extract No. 343) instead of nutrient broth. To test this possibility, one sterile egg was placed into each of twenty tubes which contained 5 ml of 0.5% solution of liver extract (Eli Lilly). By the end of the fifth day of incubation only one of the eggs had hatched. Ten of the remaining tubes, in which no hatching had occurred, were then exposed to the air for

contamination. Eighty per cent hatching occurred in the contaminated tubes by the end of the eighth day, while no further hatching occurred in the sterile tubes.

Ten eggs which were taken at the end of the fifth day from sterile tubes of nutrient broth and introduced into ten tubes of autoclaved 1% suspension of brewer's yeast (Fleischmann's) in a 0.5% solution of liver extract also failed to hatch by the end of the eighth day, as did the ten check eggs left sterile in nutrient broth.

It thus appeared that a large proportion of the eggs of the particular strain of *Aedes aegypti* used in this study do not hatch in the absence of living micro-organisms, or their products, in either nutrient broth, liver extract, or in a combination of killed yeast and liver extract. Contamination with micro-organisms, however, seems to cause a relatively high percentage of hatching.

Effect of pure cultures of fungi.—Pure cultures of several species of micro-organisms were next tested for comparative effect on the hatching of eggs. It was thought that a comparison and contrasting of the properties of the organisms might enable one to determine which properties influence hatching. The eggs used in these tests had been lying dormant in sterile nutrient broth for five days. Some of these were left undisturbed as controls. Inspection of Table 1 reveals that all of the micro-organisms tested are capable of stimulating the hatching of the eggs. This effect is exerted for the most part during the first twenty-four hour period of fungal growth. The fact that the hatching of the eggs in the presence of *Sarcina lutea* occurred somewhat later than in other cases may be explained by the relatively slow growth rate of this organism.

TABLE 1.—The effect of various known bacteria, yeast, and molds on the hatching of dormant eggs of *Aedes aegypti*

Fungi	No. of eggs, 1 per tube	Number hatched					% hatched by 60th hour
		12 hrs.	24 hrs.	36 hrs.	48 hrs.	60 hrs.	
<i>Bacillus subtilis</i>	30	28	2	100
<i>Escherichia coli</i>	30	28	2	100
<i>Staphylococcus aureus</i>	30	22	3	0	0	0	83
<i>S. albus</i>	30	22	5	0	0	0	90
<i>S. citreus</i>	30	18	10	0	0	0	93
<i>Sarcina lutea</i>	30	0	11	13	1	1	87
<i>Serratia marcescens</i>	30	27	0	0	0	0	90
<i>Pseudomonas aeruginosa</i> ..	30	26	1	0	0	0	90
<i>Saccharomyces cervisiae</i> ..	30	15	4	0	0	0	63
<i>Aspergillus niger</i>	30	23	0	5	1	0	97
<i>Penicillium</i> sp.	30	10	16	0	0	0	87
<i>Rhizopus nigricans</i>	30	24	0	0	0	0	80
<i>Fusarium moniliforme</i>	30	25	2	0	0	0	90
<i>Glomerella grossypii</i>	30	14	12	0	0	0	87
Combined controls	120	0	0	0	0	0	00

Effect of crowding.—One essential difference between the technique described above and that used by Trager (1935) lies in the number of eggs placed for hatching in each culture tube. Trager placed from fifty to two hundred in each culture, whereas the eggs were placed singly in the above experiments. To test for the possible effect of crowding on hatching, therefore, various numbers of eggs were placed together in the culture tubes. Table 2 gives the number of eggs in each tube and the percentage of hatching which occurred in each case. It should be noted that the eggs in fifty-one of the tubes which contained fifty-three or more eggs were collected during a forty-eight hour period, and were dried for eight days prior to surface sterilization.

TABLE 2.—The effect of crowding on the hatching of the eggs under sterile conditions

Eggs per tube	No. of tubes	Total no. of eggs	Total no. hatched by 5th day	% hatched
1	113	113	7	6
2	21	42	9	21
3-6	24	98	18	19
7-11	9	68	31	47
14-23	5	97	50	52
37-51	2	88	46	53
53	1	53	44	83
58	1	58	47	83
63	1	63	53	84
64	1	64	51	80
74	1	74	44	59
119	1	119	87	73

Thus it appears that the crowding of the eggs under sterile conditions produces a higher percentage of hatching than occurs when only one egg is contained in the culture tube, but still not as high a percentage as is produced by the concomitant growth of micro-organisms (Table 1).

The way in which the growth of micro-organisms and the simple crowding of the eggs promote hatching of the eggs remains an enigma. Two possibilities which seem to merit further consideration as factors involved in the initiation of the hatching process are the possible increase in the permeability of the egg wall to water and the change in the oxidation-reduction potential of the medium. Further studies regarding these possibilities are planned.

SUMMARY

1. White's solution as used by Trager (1937) was found to be 100% effective in the surface-sterilization of the eggs of *Aedes aegypti*.

2. *Aedes aegypti* which were cultured singly in five ml of sterile media (nutrient broth, or liver extract and killed yeast) rarely hatched.

3. Pure cultures of the following heterotrophic fungi were found to stimulate the hatching of dormant eggs: *Escherichia coli*; *Sarcina lutea*; *Pseudomonas aeruginosa*; *Serratia marcescens*; *Saccharomyces cerevisiae*; *Aspergillus niger*; *Penicillium* sp.; *Rhizopus nigricans*; *Fusarium moniliforme*; *Glomerella grossypii*. Since all of these organisms produced a relatively high percentage of hatching in the eggs, it seems that this property is generally distributed among heterotrophic fungi. This substantiates the observations by Atkin and Bacot (1917) and Roubaud and Colas-Belcour (1927).

4. When eggs were crowded together in the cultures a relatively large percentage hatched, though not as large a percentage as when micro-organisms were introduced. This stimulation of hatching due to simple crowding may possibly explain why Barber (1928), MacGregor (1929), Hinman (1930), Rozeboom (1935) and Trager (1935, 1937) did not discover any relationship between the presence of living micro-organisms and the hatching of *Aedes aegypti* eggs.

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FLOWERS AS A SUGGESTED SOURCE OF MOSQUITOES DURING ENCEPHALITIS STUDIES, AND INCIDENTAL MOSQUITO RECORDS IN THE DAKOTAS IN 1941*

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Incident to late season studies made in North and South Dakota in connection with the 1941 outbreak of encephalitis, it was found that numbers of certain species of mosquitoes desired for tests for encephalitis virus could be obtained as readily from flowers as by general collecting from animals, roosts, and the like. The observations are reported as suggestive of the possible importance of flowers as a supplemental source of mosquitoes for test use.

It was presumed that most flower-visiting mosquitoes would be males and recently emerged females of little value as possible virus carriers because mostly unfed. Therefore, it was somewhat of a surprise to discover that certain flowers such as goldenrod (*Solidago*) were visited extensively, not only by males and young females, but also by females with blood meals in various states of digestion and even by some with abdomens swollen with eggs. Visitation was both diurnal and nocturnal. Among the species collected from goldenrod were *Aedes vexans*, *spencerii*, and *campestris*, *Culex tarsalis* and *territans*, and *Theobaldia inornata*.

It was also noted on several occasions in the late afternoon, that, unless there was a freshly emerged "crop" of *Aedes* due to recent rains, the bulk of the females (including the older ones that were more desirable from a test-standpoint) actually seemed to prefer the goldenrod blossoms to attacks on persons nearby. This facilitated collecting with an aspirator. Even in the late part of the season as the night temperatures approached freezing, the insects could still be taken by flashlight on the younger blossoms. This was also true in wet, cool weather, when collections elsewhere were mostly discouraging. For example, 3♂, 2♀ of *C. tarsalis*, 3♀ of *T. inornata* and 2♀ *A. vexans* were taken on goldenrod between 10:30 and 11:00 PM, September 9, at Webster, South Dakota, after a drop to 38° F from an afternoon temperature of 64° F.

Aedes spencerii, *C. tarsalis*, *T. inornata*, and *A. vexans* were found on flowers in that order of abundance, the females outnumbering the males. Among the important biters encountered in the area, only the anophelines were conspicuously

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absent. Only one female each of *A. maculipennis* at Rugby, North Dakota, and *A. campestris* at Pelican Rapids, Minnesota, were found on flowers.

Among incidental observations, it also may be mentioned that *A. maculipennis* was found in some abundance (not on flowers) in certain localities in central and eastern North Dakota, and in northeastern South Dakota. *A. punctipennis* was less common, but of special interest was the observed occurrence of *A. quadrimaculatus* at Valley City. One of two specimens seen was captured. The identification was confirmed by Dr. T. H. G. Atken. The farthest previous northwest record of this important southern malarial vector is for eastern Minnesota, so this observation extends the distribution for a considerable distance into the Northern Plains area.

Among other observations of interest was the taking of a freshly fed specimen each of *A. maculipennis* and *C. tarsalis* in a chicken house at Towner, N. Dak. Several *C. pipiens* were captured at Waubay and Brookings, S. Dak., while *C. territans* as well as *C. tarsalis* were found breeding at several locations in North Dakota west to the vicinity of Rugby. In the earlier samples, *Aedes vexans* and *A. dorsalis* were the most abundant mosquitoes taken on domestic stock, but a mid-September rain resulted in emergence in countless numbers of a late brood of *A. spencerii* which then became the dominant pest of man and animals by September 22. This observation of a late summer brood (or at least a delayed hatching) may be of some consequence since both *A. idahoensis* and *A. spencerii* (which Matheson suggests as possibly the same) are reported in the literature as having only one annual spring brood which hatches from eggs laid the previous year, but the adults are reported to persist through the season into September. Specimens of both sexes were submitted to Dr. W. B. Owen who confirmed their identification.

LARVAL TREMATODES OF NORTHWEST IOWA. I. NINE NEW XIPHIDIOCERCARIAE¹

F. G. BROOKS

Studies on larval trematodes of the Okoboji region pursued by the writer during the summers of 1940, 1941, and 1942 have revealed a number of new forms. In the present paper nine stylet cercariae will be described.

METHODS OF STUDY

Snails were collected from the lakes, sloughs, and kettleholes that abound in the region. The usual procedure of isolating the snails in half-pint milk bottles was used. The cercariae were studied alive, both unstained and stained with various intra-vitam dyes of which neutral red and Nile blue sulfate proved to be most useful.

Measurements were taken on living larvae and on specimens killed in several ways. The method of heating them in a drop of water on a slide without a cover-slip seemed to produce results most in keeping with the measurements on the living larvae. The slide would be laid on an electric hotplate for five seconds. A second method was to centrifuge the contents of the milk bottle and, after taking up the material from the bottom of the tube with a pipette, squirting it into hot 10% formalin. This caused the cercariae to shrink considerably, but gave rather uniform results. For some forms both methods for killing were used, especially when it expedited the comparison with related cercariae. Averages of from ten to twenty measurements were usually taken.

The writer has been impressed with the uniformity of the dimensions of the stylets of the various species of xiphidiocercariae studied. He believes that greater use can be made both of the shape and length of the stylet in describing and in identifying cercariae of this group. He found no significant aberration in shape among the various specimens of any species studied. The fluctuation in length did not exceed one micron and seldom was any difference noticeable beyond the limitations of the ordinary micrometer. In this study, complete dependence was not placed on either the length or the shape of the stylet in distinguishing species, but such differences did corroborate determinations made on other grounds.

Cercaria lajeae n. sp. (Figs. 1, 2)

Specific diagnosis: Xiphidiocercaria of Armatae group characterized by narrow body and conspicuous cornuae which nearly encircle acetabulum. Stylet needle-shaped with reenforced sides and basal bulb; 0.024 mm in length. Seven large penetration glands on each side anterior and lateral to acetabulum. Prepharynx shorter than pharynx; ceca short. Excretory bladder with bulbous posterior chamber and large cornuated anterior vesicle which nearly surrounds acetabulum; small granules (2-5 μ) found in both vesicles; posterior vesicle empties at base of tail through excretory canal equipped with heavy sphincter muscle; flame cells not visible. Cystogenous glands conspicuous; large glands in rows along lateral edges; clusters of small glands in region of pharynx. Oil-like refractile globules present in modern quantity. Body covered with fairly heavy spines in diamond cross-hatch pattern; spines heavier at anterior end.

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¹ From the Iowa Lakeside Laboratory and Cornell College.

The writer is indebted to Professor T. C. Stephens of Morningside College, a member of the staff of Iowa Lakeside Laboratory, for the identification of the snails mentioned in this paper.

Measurements: Living cercariae range from 0.510×0.065 mm when fully extended, to 0.180×0.160 mm when contracted. Tail length ranges from 0.425 mm to 0.120 mm. Average measurements in millimeters of emerged living cercariae moderately distended and of emerged cercariae killed with heat are:

	Living	Killed with heat
Body length	0.330	0.391
Body width	0.140	0.147
Tail length	0.370	0.340
Tail width	0.034	0.048
Oral sucker length	0.061	0.066
Oral sucker width	0.063	0.076
Acetabulum diameter	0.068	0.074
Stylet length	0.024	0.024
Stylet width	0.005	0.005

Precercarial stage: *C. lajeae* develops in yellow sporocysts that cannot be separated from tissue of digestive gland of snail.

Host and Locality: Found infecting *Physella elliptica* Lea collected from kettlehole five miles southwest of Lakeside Laboratory.

C. lajeae has many points in common with *C. brevicaca* Cort, 1915, *C. ramonae* McCoy, 1928, *C. of Renifer kansenses* (Crow, 1913) described by McCoy, 1928, *C. of Lechriorchis primus* Talbot, 1933, *C. of Renifer aniarum* Byrd, 1935. *C. lajeae* differs significantly from all these forms in the shape of the stylet, by having fewer penetration glands and in having a muscular excretory canal. Since all cercariae of the genus *Renifer* whose life histories have been completed have an excretory bladder whose cornuae nearly surround the acetabulum, it is very probable that *C. lajeae* will be found to belong to this genus.

Cercaria kingi n. sp.²
(Figs. 3-5)

Specific diagnosis: An ornate xiphidiocercaria of Prima group. Tail short and stubby with dorso-ventral finfold. Stylet needle-shaped, 0.039 mm long; no basal bulb. Five pairs of penetration glands anterior and lateral to acetabulum; first two coarsely granular. Prepharynx nearly as long as esophagus; ceca extend to region of bladder. Excretory bladder with bulbous posterior chamber and v-shaped anterior vesicle; common duct of collecting tubes received terminally by cornuae; abundant cystogenous glands make determination of flame cell pattern impossible. Small, closely-set spines cover body; spines heavier at anterior end; protruding bristles (0.009 mm long) sparsely scattered over body.

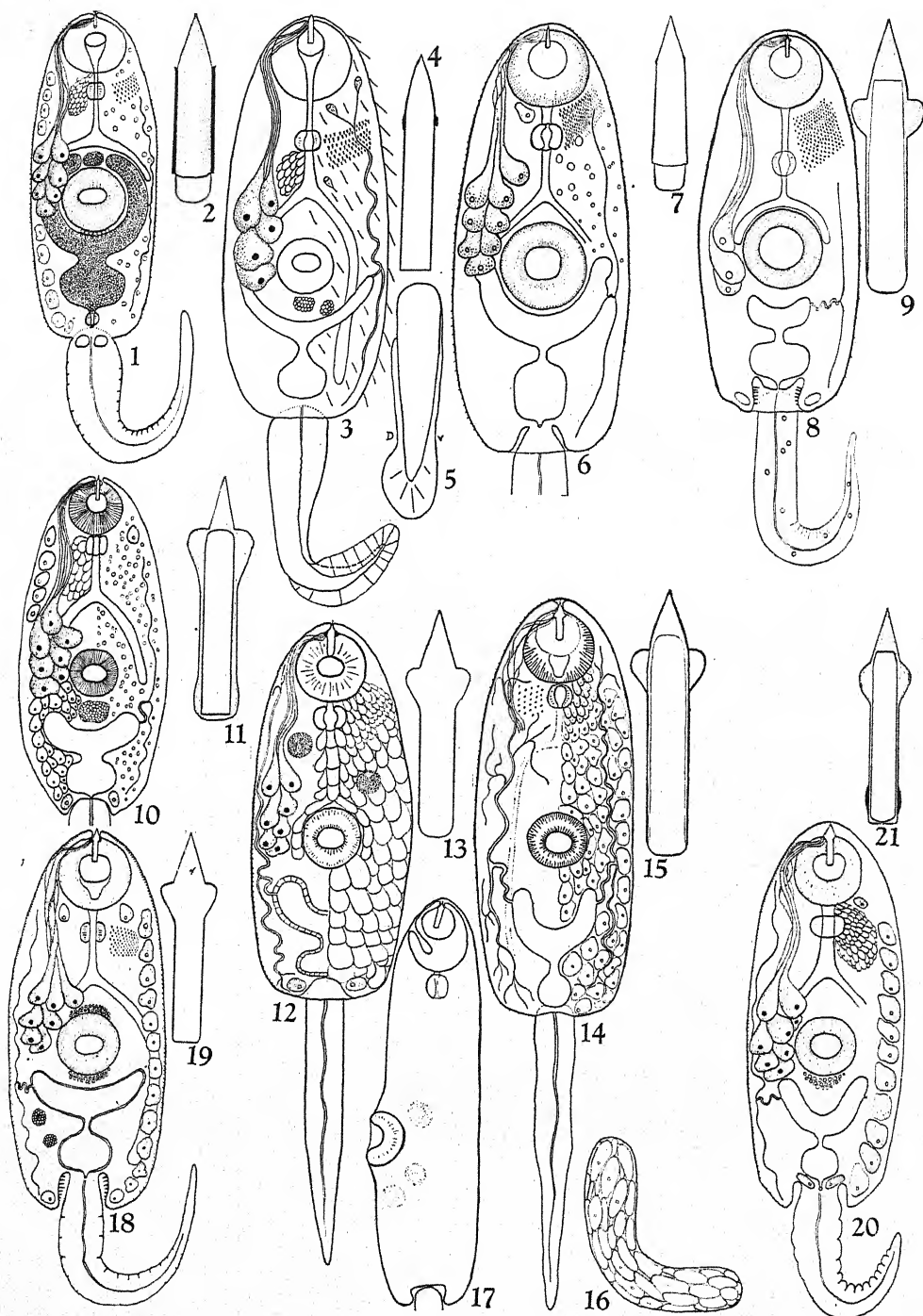
Measurements: Body is distensible, attaining maximum length of 0.370 mm; contracted length 0.155 mm. Average dimensions of living cercariae and of those killed in 10% hot formalin:

	Living	Killed in hot 10% formalin
Body length	0.270	0.240
Body width	0.125	0.110
Tail length	0.160	0.137
Tail width	0.062	0.056
Oral sucker diameter	0.071	0.062
Acetabulum diameter	0.041	0.042
Stylet length	0.039	0.039
Stylet width	0.005	0.005
Greatest width of finfold	0.019

Host and Locality: Found as parasites of *Stagnicola umbrosa* Say collected 1940 from Manhattan Slough, two miles north of Lakeside Laboratory.

Lühe (1909) created the Ornatae group of xiphidiocercariae to include all stylets with a caudal finfold. Sewell (1922) subdivided this unnatural group and included in his Prima division those median-sized forms in which the acetabulum is

² Named in honor of Professor Robert L. King.

FIG. 1. *Cercaria lajeae* n. sp.FIG. 2. Stylet of *Cercaria lajeae*.FIG. 3. *Cercaria kingi* n. sp.FIG. 4. Stylet of *Cercaria kingi*.FIG. 5. Lateral view of tail, *Cercaria kingi*.FIG. 6. *Cercaria diamondi* n. sp.FIG. 7. Stylet of *Cercaria diamondi*.FIG. 8. *Cercaria pili* n. sp.FIG. 9. Stylet of *Cercaria pili*.FIG. 10. *Cercaria coniae* n. sp.

smaller than the oral sucker; in which the tail is shorter than the body and has a dorso-ventral finfold of which the ventral portion extends farther forward; which have a prepharynx and pharynx and ceca reaching beyond the acetabulum; which have four or five penetration glands; which have an oval-shaped excretory bladder with cornuae; and which develop in oval or sack-shaped sporocysts.

C. kingi clearly belongs to the Prima group. Other members of this group are: *C. prima* Ssinitzin, 1905; *C. ornata* LaValette, 1855; *Cercariae indicæ* XXIV and XXVIII Sewell, 1922; *C. longistyla* McCoy, 1929; *C. exilis* Brooks, 1930; *C. Pneumonoeces medioplexus* and *C. Pneumobites parviplexus* Krull, 1931; and *C. merchanti* Rankin, 1939. Of these, this new form most resembles *C. longistyla* but differs from it significantly in the length and shape of the stylet.

Cercaria diamondi n. sp.
(Figs. 6, 7)

Specific diagnosis: Xiphidiocercaria of Armata group. Stylet without shoulders and with basal bulb; stylet proper 0.019 mm long, bulb 0.004 mm long, slightly narrower than base of stylet proper. Eight pairs of penetration glands anterior-lateral to acetabulum. Excretory bladder with bulbous posterior chamber and v-shaped anterior vesicle; common duct from collecting tubes received subterminally. Cystogenous glands conspicuous. Refractile globules present in moderate amount. Body covered with fine closely-set spines.

Measurements: Average measurements in millimeters of emerged cercariae killed with heat:

Body length	0.418	Pharynx length	0.023
Body width	0.160	Pharynx width	0.032
Tail length	0.337	Middle acetabulum to posterior end ..	0.169
Tail width	0.039	Stylet length without bulb	0.019
Oral sucker length	0.063	Stylet width at base	0.004
Oral sucker width	0.071	Bulb length	0.004
Acetabulum diameter	0.071	Bulb width	0.003
Prepharynx	0.025		

Pre-cercarial stage: Cercariae occur in delicate sporocysts with thin walls which cannot be separated from tissue of digestive gland of snail host.

Host and Locality: Obtained from two snails of collection of 480 specimens of *Physella elliptica* Lea collected 1942 at Diamond Lake, seven miles north of Lakeside Laboratory.

Activity.—*C. diamondi* is very active in water and continues the writhing motion characteristic of its group for a considerable time under a coverslip.

The "oily" globules and opaque cystogenous and parenchymous cells hide many finer structures. Globules of various sizes range from tiny to ovoid globules 0.009 mm long. All penetration glands stain with acid stain and are drained by four pairs of ducts whose openings can sometimes be seen at the front of the oral sucker on either side of the stylet. An occasional cercaria shows a large cell that stains heavily with neutral red near the oral sucker. The stylet lacks shoulders but shows a slight refraction of light where its sides start to taper. The basal bulb seems to be a continuation of the core of the stylet proper. In most larvae, one side of the stylet was nearly a micron longer than the other side. The stylets of the cercariae from the second snail were a micron longer than those from the first snail, but there was not that much range in the length of the stylets from either snail.

- FIG. 11. Stylet of *Cercaria coniae*.
FIG. 12. *Cercaria argenti* n. sp.
FIG. 13. Stylet of *Cercaria argenti*.
FIG. 14. *Cercaria dorotti* n. sp.
FIG. 15. Stylet of *Cercaria dorotti*.
FIG. 16. Sporocyst of *Cercaria dorotti*.

- FIG. 17. Lateral view of *Cercaria dorotti*.
FIG. 18. *Cercaria nolfi* n. sp.
FIG. 19. Stylet of *Cercaria nolfi*.
FIG. 20. *Cercaria aalbui* n. sp.
FIG. 21. Stylet of *Cercaria aalbui*.

C. diamondi resembles *C. holthauseni* Rankin, 1939, and *C. tricystica* E. L. Miller, 1935, in the number of penetration glands, type of the excretory bladder, length of stylet and in having a stylet bulb. It differs from both, however, in that the stylet is of different shape. It would seem that Rankin's form has less apparent cystogenous glands and refractile globules because he does not mention them and was able to work out the flame cell pattern. Miller's cercaria is considerably smaller than the present form.

Cercaria pili n. sp.

(Figs. 8, 9)

Specific diagnosis: Xiphidiocercaria of Armatae group. Stylet 0.020 mm long, javelin-shaped; no basal bulb. Pharynx situated more than half way between oral sucker and fork of esophagus; ceca short. Three pairs of penetration glands—two lateral to acetabulum and one slightly posterior; three ducts from penetration glands to point of stylet. Excretory bladder consists of bulbous posterior chamber and cornuolated anterior vesicle which receives the common ducts from the anterior and posterior collecting tubes subterminally. Caudal receptacle large, muscular, caudal sinuses spined. Fine spines thickly set over entire body in diamond cross-hatch pattern.

Measurements: Average measurements in millimeters of emerged cercariae killed with heat:

Body length	0.195	Oral sucker width	0.038
Body width	0.065	Acetabulum diameter	0.032
Tail length	0.128	Stylet length	0.020
Tail width	0.032	Stylet width through shaft	0.004
Oral sucker length	0.041	Stylet width across shoulders	0.006

Precercarial stage: Produced in sporocysts that are nearly white, but with flecks of yellow pigment. Thin walled, but separate rather easily from tissue of digestive gland. Nearly uniform in diameter, but variable in length; larger ones measured 0.920 long by 0.130 mm wide.

Host and Locality: Found as parasites of *Helisoma trivolvis* Say collected at Hotte's Lake, ten miles northeast of Lakeside Laboratory.

Activity.—The mature cercariae leave the snail in the early morning and are no longer found swimming at noon. They are very active in the water and on a slide they use their tails more than other forms studied.

The body contains no refractile globules, but slightly refractive inclusions are at irregular intervals along the tail. Penetration glands are easily detected, with coarse, granular material. The stylet is held pointing downward at nearly a right angle and is large for the size of the cercaria. The cystogenous glands and parenchymous cells hide the smaller structures of the excretory system, making even the collecting tubes hard to see. The anterior vesicle is not often found to be filled in mature forms. The caudal receptacle is a characteristic feature. Its strong walls hold its shape after the tail is lost and at first glance might be mistaken for the bladder.

The form most resembling *C. pili* is *C. tridena* Miller, 1936. Their size, length of stylet, nature of larger excretory organs and number of penetration glands are similar or essentially so. Essential differences are found in the position of the pharynx, color of the sporocysts and in the fact that *C. tridena* has its caudal pockets reduced and these pockets are without spines in their walls.

Cercaria conmieae n. sp.

(Figs. 10, 11)

Specific diagnosis: Xiphidiocercaria of the Armatae group. Stylet javelin-shaped, 0.029 mm long; shaft reenforced at base, no basal bulb. Prepharynx practically absent, pharynx well developed; esophagus forks well above acetabulum; ceca do not extend beyond posterior of

acetabulum. Seven pairs of penetration glands anterior and lateral to acetabulum drained by four pairs of ducts. Bladder with bulbous posterior chamber and v-shaped anterior vesicle; pair of short tubules leave cornuae subterminally to receive anterior and posterior collecting tubes. Dense cystogenous glands and abundant large oil-like globules obscure flame cells. Body spines short and sparsely distributed.

Measurements: Average dimensions in millimeters of moderately distended living cercariae and of cercariae killed in hot 10% formalin:

	Living	Killed in hot 10% formalin
Body length	0.270	0.158
Body width	0.128	0.092
Tail length	0.115	0.094
Tail width	0.040	0.026
Oral sucker, diameter	0.046	0.041
Acetabulum, diameter	0.036	0.029
Distance acetabulum to posterior end	0.045
Diameter larger oil-like globules	0.006
Stylet length	0.029	0.029
Stylet width across shaft	0.004	0.004
Stylet width across shoulder	0.007	0.007

Pre cercarial stage: Development occurs in long, tube-like sporocysts with small amount of golden pigment in walls.

Host and Locality: Found as parasites of *Stagnicola palustris elodes* Say collected 1940 from West Silver Lake Bog, ten miles northwest of Lakeside Laboratory.

Since this and the next four closely related cercariae seem to form a natural subgroup, their taxonomic relations will be discussed after they have all been described.

Cercaria argenti n. sp.
(Figs. 12, 13)

Specific diagnosis: Xiphidiocercaria of Armatae group. Stylet javelin-shaped with large shoulders; 0.032 mm long; no basal bulb. Body well supplied with cystogenous glands which give whitish luster. Refractile globules present. Seven pairs of penetration glands anterior and lateral to acetabulum. Prepharynx very short, not visible when larva is not extended. Esophagus and ceca difficult to distinguish. Excretory system consists of posterior vesicle which joins v-shaped anterior vesicle by narrow canal; both vesicles and canal with thick walls; main collecting tubes join cornuae subterminally. Very fine spines form diamond cross-hatch pattern over entire body, more conspicuous on ventral side and at anterior end. Small spines in caudal sinuses.

Measurements: Average measurements in millimeters of emerged living cercariae and of those killed with heat:

	Living	Killed with heat
Body length	0.340	0.315
Body width	0.144	0.124
Tail length	0.186	0.178
Tail width	0.038	0.035
Oral sucker length	0.068	0.062
Oral sucker width	0.066	0.060
Acetabulum diameter	0.049	0.047
Pharynx length	0.022
Pharynx width	0.020
Stylet length	0.032
Stylet width across shaft	0.005	0.005
Stylet width across shoulder	0.008	0.008

Pre cercarial stage: Sporocysts are short, thin-walled structures that do not separate readily from tissue of digestive gland.

Host and Locality: *Cercaria argenti* was found in 1940 infecting *Stagnicola palustris elodes* Say collected from West Silver Lake Bog, and, in 1942, in ten snails from collection of 620 of *Stagnicola umbrosa* Say collected from Stony Lake, eleven miles west of Lakeside Laboratory.

This cercaria is characterized by a great abundance of cystogenous glands which make most other structures difficult to distinguish. These glands are most dense on the ventral side. Refractile globules are present, but small and not so abundant as to hide other structures. The esophagus and ceca are effectively hidden by rows of small glands. The seven penetration glands are smaller than the average for closely related forms. Four ducts lead from these glands and empty near the base of the shoulders of the stylet. Two specimens contained round, glandular bodies which stood out quite distinctly from the surrounding structures. In each case one was located on the right side of the body just in front of the acetabulum while the other was on the left, farther front and more to the side. The cercariae react positively to light. They are vigorous; not only do they swim actively when free in water, but they maintain their characteristic "measuring worm" movement for a longer time than is usual for the other stylets after they have been placed on a slide.

It is worthy of note that the stylets of several hundred cercariae from eleven different snails of two species did not vary as much as a micron in length.

Cercaria dorotti n. sp.
(Figs. 14-17)

Specific diagnosis: Xiphidiocercaria of Armatae group characterized by very dense cystogenous glands. Stylet javelin-shaped; 0.034 mm long; no basal bulb. Penetration glands not determined, but five ducts can be seen to empty at base of stylet. Prepharynx very short; esophagus and ceca not seen in emerged specimens. Excretory bladder consists of bulbous posterior chamber and v-shaped anterior vesicle which receives common duct from collecting tubes subterminally. Very small refractile globules present, located principally at edges of cystogenous glands. Body covered by fine spines arranged in diamond cross-hatch pattern.

Measurements: Average measurements in millimeters of emerged living cercariae of medium contraction, of specimens killed by heat, and of those killed in hot 10% formalin:

	Living	Killed with heat	Killed in 10% formalin
Body length	0.280	0.314	0.237
Body width	0.115	0.120	0.102
Tail length	0.184	0.192	0.124
Tail width	0.034	0.034	0.031
Oral sucker length	0.066	0.058	0.048
Oral sucker width	0.066	0.060	0.049
Acetabulum diameter	0.045	0.041	0.033
Pharynx length	0.027	0.020	0.018
Pharynx width	0.021	0.024	0.018
Stylet length	0.034	0.034	0.034
Stylet width through shaft	0.005	0.005	0.005
Stylet width across shoulder	0.008	0.008	0.008

Preercarial stage: Sporocysts are small, orange-colored, sausage-shaped structures with blunt, rounded ends. Thickly packed in digestive gland of host from which they did not separate readily. Typical sporocysts measured from 0.9 to 1.2 mm in length and 0.170 to 0.210 mm in width.

Host and Locality: Found in single snail of *Stagnicola umbrosa* Say collected 1942 from Manhattan Slough, two miles north of Lakeside Laboratory.

Activity.—These cercariae emerge in the morning and spend several hours swimming actively. They are usually seen in swarms which may be found in any part of the bottle. They are neither attracted nor repelled by light of moderate intensity.

A study of immature cercariae revealed certain structures that were entirely hidden by the dense cystogenous glands of emerged specimens. The penetration

glands were found to be about the same size as the cystogenous glands, therefore, small, and at least five in number on each side. The esophagus was found to fork well above the acetabulum and the ceca to extend to the region of the bladder. A few immature cercariae showed the collecting tubes of the excretory system and their accessory branches very plainly. Each tube had three accessory tubules each of which gave off three capillary tubules. Thus there are evidently eighteen flame cells on each side, though none were seen. An immature cercaria studied in side view showed three nearly spherical bodies which were taken to be parts of the reproductive primordium and are shown in Fig. 17. Two bodies posterior to the acetabulum are evidently the testes while a large body above the anterior edge of the acetabulum may be either the primordium of the metraterm or of the cirrus or of both those structures.

Cercaria nolfi n. sp.³

(Figs. 18, 19)

Specific diagnosis: Xiphidiocercaria of Armatae group made opaque by abundant cystogenous glands and refractile globules. Stylet javelin-shaped with strong shoulders, 0.034 mm long; no basal bulb. Seven or eight penetration glands located anterior and lateral to acetabulum. Prepharynx short; esophagus forks short distance above acetabulum; ceca extend to bladder. Excretory bladder consists of bulbous posterior chamber and v-shaped anterior vesicle. Row of about twelve large cystogenous glands extends along each lateral edge of larva and smaller glands fill in solidly between; of these, a pair in each posterior lappet and at posterior-lateral edge of oral sucker are larger and more apparent. Body covered with fine spines in diamond cross-hatch pattern. Spines present in caudal sinuses. Reproductive fundament consists of cells in front of and behind acetabulum and two groups of cells lateral to opening between anterior and posterior vesicles.

Measurements: Average measurements in millimeters of emerged cercariae killed with heat:

Body length	0.328	Prepharynx	0.022
Body width	0.136	Pharynx length	0.024
Tail length	0.185	Pharynx width	0.027
Tail width	0.039	Stylet length	0.034
Oral sucker diameter ..	0.063	Stylet width through shaft	0.005
Acetabulum	0.051	Stylet width through shoulders ..	0.008

Precercarial stage: Sporocysts are long, orange-colored structures occurring in tangled masses. They are 0.350 mm in diameter and exceed one millimeter in length.

Host and Locality: Found as parasites of *Stagnicola palustris elodes* Say collected 1941 at West Silver Lake Bog and of *Stagnicola umbrosa* Say from Stony Lake.

Cercaria aalbui n. sp.

(Figs. 20, 21)

Specific diagnosis: Xiphidiocercaria of Armatae group. Stylet javelin-shaped but with slight ringed enlargement near posterior end; no basal bulb; 0.038 mm in length. Eight penetration glands anterior and lateral to acetabulum empty through four ducts which tend to become bulbous near oral sucker. Prepharynx short, pharynx broad, ceca cannot be distinguished below acetabulum. Excretory bladder with bulbous posterior chamber and v-shaped anterior vesicle whose cornuae receive common ducts from collecting tubes subterminally. Row of about twelve large cystogenous glands along each lateral edge; other glands of smaller size abundant especially in esophageal region; a gland in each posterior lappet and at each posterior-lateral edge of oral sucker are more noticeable than others. Refractile globules large and abundant, more numerous on dorsal side. Body covered by fine spines in diamond cross-hatch pattern, heavier toward anterior end; no spines in caudal sinuses.

Measurements: Living cercariae vary from 0.408×0.054 mm well extended to 0.170×0.140 mm contracted. Tail varies from 0.250 mm in length extended to 0.092 mm contracted. Average measurements in millimeters of emerged living cercariae and of those killed by heat:

³ This species is named in honor of Professor L. O. Nolf.

	Living	Killed with heat
Body length	0.348	0.337
Body width	0.152	0.135
Tail length	0.216	0.225
Tail width near base	0.040	0.038
Oral sucker length	0.058	0.065
Oral sucker width	0.063	0.070
Acetabulum diameter	0.044	0.052
Pharynx length	0.018
Pharynx width	0.021
Stylet length	0.034	0.034
Stylet width across shaft	0.005	0.005
Stylet width across shoulders ..	0.007	0.007

Precercarial stage: Long yellow sporocysts occur in tight, tangled masses; old sporocysts become rusty colored; typical size of sporocyst 1.2 mm long by 0.27 mm in diameter.

Hosts and Locality: *C. aalbui* was found infecting numerous specimens of *Stagnicola palustris clodes* Say from several collecting grounds in 1940, 1941, and 1942; from *S. umbrosa* Say collected from Green Bay Bog in 1940, and from same species collected from Stony Lake in 1942.

Activity.—The swimming movements and behavior under a cover slip were typical for the group except that the tail of this cercaria seems to have adhesive qualities and the larva uses it for sticking to the substratum or any objects it touches. No glands were found to account for its adhesiveness. Martin, 1939, speaks of the cercaria of *Stephanostomum tenue* using its tail in similar fashion, but he likewise found no caudal adhesive glands.

C. conniae, *C. argenti*, *C. dorotti*, *C. nolfi*, and *C. aalbui*, have so many structural features in common that they seem to form a natural sub-group. The most striking similarities pertain to the stylet. In every case it is large, javelin-shaped, and does not have a basal bulb. The size of the shoulders varies a little, being heaviest in *C. argenti*, *C. dorotti*, and *C. nolfi*. In *C. conniae* and *C. aalbui* the wall is slightly thickened near the base. With the exception of these minor differences and the differences in length, the stylets of all five forms are very similar. Other similarities pertain to the general size and shape of body and tail, size of oral sucker and acetabulum and their dimensional ratio, the number and position of the penetration glands, the shape of the excretory bladder, and the spination.

There are significant differences, however, among them. *C. conniae* and *C. aalbui* are the only cercariae of the group that have a ring near the base of the stylet. They can be distinguished from each other by the abundance of refractile (i.e. oil-like) globules so apparent in *C. conniae* and by the larger size of *C. aalbui*. *C. argenti* has been found oftener than any of the five forms. It is characterized by the sheen of its cystogenous glands and their adhesion to the digestive system. *C. dorotti* is the only one in which the cystogenous glands completely hide the digestive system in emerged forms and make impossible the determination of the number and position of the penetration glands. *C. nolfi* has a large caudal receptacle with spines in the caudal sinuses. It is the only one to possess this trait. Cort (1915) created the sub-group Polyadena to include cercariae possessing the following characters:

1. Development in gastropods in elongate sac-shaped sporocysts.
2. Tail slender and less than the body length except when very much extended.
3. Acetabulum back of the middle of the body and smaller than the oral sucker.
4. Stylet about 0.30 mm in length, six times as long as broad, and with a thickening one-third of the distance from the point to the base.

5. Stylet glands, six or more on each side between the acetabulum and the pharynx.

6. Excretory bladder bicornuate.

7. Very short prepharynx and small pharynx present. Esophagus when developed short to of medium length. Intestinal ceca (when present) reaching to posterior end of body.

The five members of this group meet Cort's specifications except that the ceca of *C. conniae* and *C. argenti* do not reach to the posterior end of the body. All five resemble *C. isocotylea* in many respects, but differ from it both in the shape of the stylet and by having apparent cystogenous glands. Of previously described members of the Polyadena group they most resemble *C. acanthocoela* Miller, 1935, but differ from it in the exact size and shape of the stylet and in the occurrence together of spined caudal sinuses and short ceca.

It is proposed that the Conniae division of the Polyadena group be erected to include cercariae with javelin-shaped stylets without basal bulbs. It is believed by the author that life history studies will prove these cercariae to be closely related.

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LARVAL TREMATODES OF NORTHWEST IOWA. II. FOUR NEW STRIGEIDS¹

F. G. BROOKS

A number of new species of cercaria have been discovered by the author since he began his studies on the larval trematodes of the Iowa lakes region in 1940. In this region, which lies in the extreme northwestern part of the state, conditions are especially favorable for an abundant trematode fauna. There are many lakes, sloughs, and kettleholes of glacial origin. The region is on the flyways of migratory birds and is the nesting ground for many water and shore birds. The non-avian vertebrate life is likewise varied and plentiful.

The methods of handling and studying the cercariae did not differ greatly from those usually employed by workers in the field. Killing for measurement was done in hot 10% formalin and if a comparison was to be made with a related form previously described by another author, measurements were also taken by the same methods used by the other author if material was available at the time the comparison was being made.

In the present paper four new strigeids will be described and a taxonomic problem in connection with one of them will be discussed.

Cercaria stephensi n. sp.²

(Figs. 1, 2)

Specific diagnosis: Furcocercaria of Strigeid group. Four pairs of small penetration glands located lateral to acetabulum and in front of its mid-line. Prepharynx and pharynx about same length; esophagus forks well above acetabulum; ceca extend to region of bladder. No commissure in excretory system. Unpigmented "eyespot" lateral to fork of esophagus. Acetabulum muscular and protrusive. About thirty forward-pointing spines form three irregular rows in circumoral spineless area; oral cap covers nearly half of anterior organ; body thickly set with fairly heavy spines not in rows; spines in region of bladder and in front of acetabulum fewer but larger, resembling bristles; four rows of spines around opening of acetabulum; furcae bear spines in six longitudinal rows; tail-stem spineless but with flagelllets; one pair of lateral body bristles. No caudal bodies; striated muscle fibers and cell nuclei show plainly in tail stem; no furcal finfolds.

Measurements: Measurements in millimeters of emerged living cercariae and of cercariae killed in hot 10% formalin:

	Living			Killed in 10% hot formalin		
	Extended	Contracted	Relaxed	Max.	Min.	Mean
Body length	0.300	0.114	0.184	0.210	0.140	0.177
Body width	0.042	0.090	0.054	0.044	0.076	0.056
Tail-stem length	0.240	0.182	0.196	0.216	0.124	0.180
Tail-stem width	0.040	0.040	0.040	0.040	0.036	0.038
Furcae length	0.288	0.220	0.238	0.192
Anterior organ length	0.048	0.032	0.038	0.045	0.036	0.038
Anterior organ width	0.032	0.032	0.032	0.025	0.020	0.024
Acetabulum diameter	0.036	0.038	0.028	0.032

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The author is indebted to Professor T. C. Stephens of Morningside College, a member of the staff of Iowa Lakeside Laboratory, for the identification of the snails mentioned in this paper.

² Named in honor of Professor T. C. Stephens of Morningside College and the Lakeside Laboratory staff.

Precercarial stage: Cercariae produced in long, white sporocysts with straight sides. Attain length of more than six millimeters and are approximately 0.150 mm in diameter. There is a birthpore near the anterior end.

Host and Locality: *C. stephensi* was found in five of 94 snails of *Lymnaea stagnalis jugularis* collected at Rush Lake, 34 miles southeast of Lakeside Laboratory.

Activity.—This is a very active cercaria. In the water it vibrates its body rapidly and under a cover-slip it flexes itself laterally, practically forming a loop. This motion seems to aid it in losing its tail, which it disposes of rather quickly.

Cercaria leplei n. sp.³

(Figs. 3, 4)

Specific diagnosis: Strigeid cercaria with both body and furcae shorter than tail-stem. Two pairs of penetration glands which do not reach beyond midline of acetabulum. "Unpigmented eyespots" at sides of body just in front of penetration glands. Prepharynx short; ceca extend a short distance beyond acetabulum. Tail stem without caudal bodies or other prominent features; no furcal finfolds. Excretory system with commissure anterior to acetabulum. Two pairs of flame cells on each side empty into anterior and two pairs into posterior collecting tubes; one pair of flame cells in proximal part of tail. Oral cap composed of seven or eight rows of heavy spines which give way to lighter spines covering head organ; fine spines sparsely distributed over body; none observed on tail-stem, or furcae; three rows of heavy spines surround opening of acetabulum.

Measurements: Average measurements in millimeters taken from emerged cercariae killed in hot 10% formalin:

Body length	0.172	Oral sucker width	0.028
Body width	0.036	Acetabulum diameter	0.022
Tail-stem length	0.194	Pharynx length	0.012
Tail-stem width	0.033	Pharynx width	0.014
Furcal length	0.182	Middle acetabulum to posterior end ..	0.042
Oral sucker length	0.034		

Precercarial stage: Sporocysts long white tubes that superficially resemble threads. Longest measured was 7 mm. Parts of it empty measured 0.05 mm, while sections crowded with embryos were 0.08 mm in diameter. Birth pore occurs 0.07 mm from anterior end.

Host and Locality: Parasite of *Physella elliptica* Lea collected at Diamond Lake, ten miles north of Lakeside Laboratory.

The relation of *C. leplei* to other species will be discussed together with that of *C. stonii* the description of which follows.

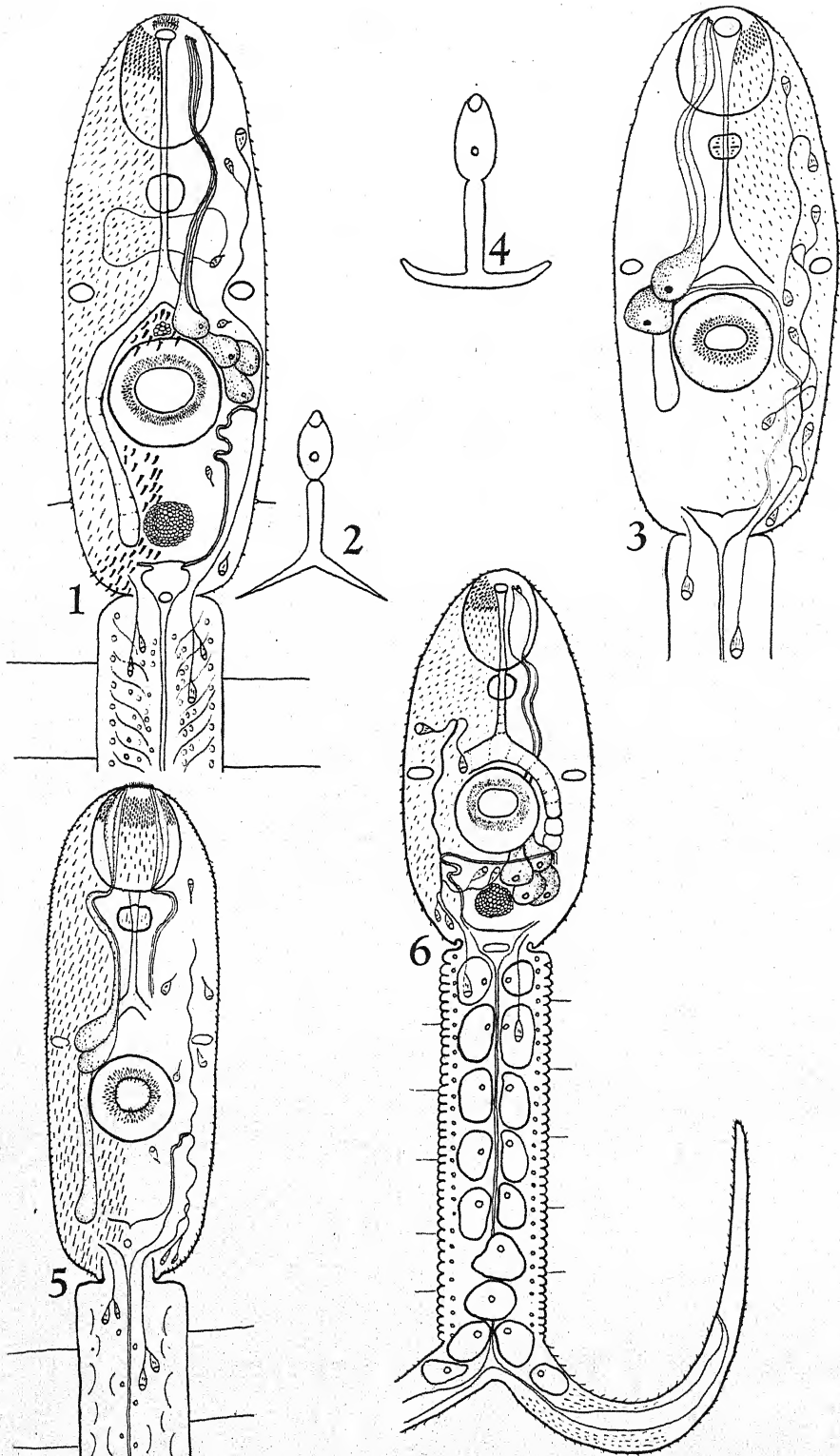
Cercaria stonii n. sp.

(Fig. 5)

Specific diagnosis: Strigeid cercaria with body and furcae slightly longer than tail-stem; oral sucker a little larger than acetabulum. Two pairs of small penetration glands antero-lateral to acetabulum. Prepharynx short; esophagus and ceca easily seen; ceca extend to region of bladder. Unpigmented "eyesps" on each side near region of acetabulum. Two rows of forward-pointing spines of about ten spines each; oral hood of eight or nine rows; body and furcae covered with fairly long spines, not in rows; three or four rows of heavy spines surround opening of the acetabulum; flagellates on tail-stem. Excretory system without commissure; two pairs of flame cells in anterior third of stem. No caudal bodies or other distinguishing features in tail-stem or furcae.

Measurements: Bodies of living cercariae may attain length of 0.22 mm at extreme extension and may contract to 0.11 mm. Length of body, tail-stem, and furcae of moderately extended living cercariae measure 160 mm, 170 mm, and 210 mm. Average measurements in millimeters of emerged cercariae killed in hot 10% formalin:

³ Named in honor of Mr. Charles Lepley of the Iowa Conservation Service.



Body length	0.196	Oral sucker length	0.037
Body width	0.058	Oral sucker width	0.031
Tail length	0.185	Acetabulum diameter	0.034
Tail width	0.032	Distance middle acetabulum to posterior end	0.066
Furcal length	0.198		

Precercarial stage: Sporocysts are long white tubes that separate readily from digestive gland of host. Have some power of movement, writhing when free in water. Large sporocysts measure 5 mm long by 0.18 mm in diameter; can be stretched to five times their normal length.

Host and Locality: *C. stonii* was found as parasite of *Lymnaea stagnalis jugularis*, collected in 1942 from Stony Lake, eleven miles west of Lakeside Laboratory.

Activity.—The activity of this cercaria when free in water is typical of strigeids. Under a cover slip it is not so active as most members of its group, showing a tendency to anchor with its acetabulum and stay in place.

The flame cell pattern could not be worked out. Fig. 5 shows eight found in the body. The flame cell near the anterior end and the one just posterior to the acetabulum are each probably one of a pair. If this is the case, the flame cell pattern would be $2[(2+2+2) + (2+2) + 2] = 24$.

C. leplei and *C. stonii* resemble each other in having two pairs of penetration glands anterio-lateral to the acetabulum. They differ in that *C. leplei* has a pre-acetabular excretory commissure while *C. stonii* is without an excretory commissure; *C. leplei* lacks forward pointing spines which *C. stonii* has; *C. leplei* has one pair of caudal flame cells while *C. stonii* has two pairs; and in that the ceca of *C. leplei* are much shorter than those of *C. stonii*.

Other strigeids without caudal bodies and having two anterio-lateral penetration glands and a preacetabular excretory commissure include *C. douglasi* Cort, 1917, *C. flabelliformis* Faust, 1917 (see Olivier and Cort, 1941), *C. sanjuanensis* H. M. Miller, 1927, *C. Strigeae tardae* Steenstrup (Mathias), 1922 (see Wesenberg-Lund, 1934, p. 117), *C. Cotylurus cornutus* Cercaria A of Szidat, 1923, and *C. fissicauda* LaVallette, 1855 (Brown, 1926). These differ from *C. leplei* by having two pairs of caudal flame cells and in various other features.

Other strigeids without caudal bodies having two anterio-lateral penetration glands and no excretory commissure include *C. marciae* LaRue, 1917 (Cort and Brooks, 1928), *C. Neodiplostomum lucidum* LaRue and Bosma (West, 1935), *C. Pharyngostomum cordatum* Wallace, 1939, *C. Alaria mustelae* Bosma, 1934, *C. Alaria intermedia* Odlaug, 1940, *C. sincera* Olivier, 1941, *C. Fibricola texensis* Chandler, 1942, *C. indicae* I Sewell, 1922, and *C. tropicalis* Faust and Hoffman, 1934. Of these *C. Neodiplostomum lucidum*, *C. Pharyngostomum cordatum*, *C. sincera*, and *C. Fibricola texensis* differ from *C. stonii* by having only one pair of caudal flame cells; *C. marciae* and *C. Alaria mustelae* lack forward-pointing spines and are much smaller; *C. Alaria intermedia* lacks unpigmented "eyespot," has shorter ceca and a differently shaped bladder.

Cercaria okobojensis n. sp.

(Fig. 6)

Specific diagnosis: Furcocercous cercaria of strigeid group. Prepharynx short; last two cells of ceca septate; ceca extend only short distance beyond acetabulum. Four pairs penetration

FIG. 1. *Cercaria stephensi* n. sp.

FIG. 2. *Cercaria stephensi* showing characteristic position of furcae.

FIG. 3. *Cercaria leplei* n. sp.

FIG. 4. *Cercaria leplei* showing characteristic position of furcae.

FIG. 5. *Cercaria stonii* n. sp.

FIG. 6. *Cercaria okobojensis* n. sp.

glands, all posterior to acetabulum, drained by two pairs of ducts which tend to form very large bulbs in anterior organ. Unpigmented "eyespot" near margin of body at level of anterior end of acetabulum. Oral cap covers less than half of anterior organ; no forward-pointing spines; body spines not in rows; furcal spines heavy, in eight longitudinal rows; four rows of prominent spines around acetabulum openings. Excretory system with post-acetabular commissure; six pairs of flame cells in body and one pair in tail-stem. Island of Cort short and broad. The two branches of excretory tubes which extend into furcae have tendency to become distended and bulbous. Fourteen distinct caudal bodies in tail-stem plus one large adjacent pair in furcae; largest caudal bodies measure about 30 by 22 microns, each with distinct nucleus. Flagellates about half as long as those seen in most strigeids, discernible in only a few specimens.

Measurements: Average measurements in millimeters of emerged cercariae taken in four different ways:

	Hot 10% formalin	Killed with heat	Mounted in balsam	Living
Body length	0.118	0.142	0.960	0.148
Body length extended	0.128	0.220
Body length contracted	0.080	0.080
Body width	0.047	0.560	0.036	0.056
Body width extended	0.028	0.310
Body width contracted	0.045	0.720
Tail-stem length	0.124	0.146	0.112	0.152
Tail-stem length extended	0.120	0.182
Tail-stem length contracted	0.105	0.094
Tail-stem width	0.036	0.030	0.038
Furcal length	0.137	0.170	0.130	0.172
Anterior organ length	0.029	0.036	0.026	0.036
Anterior organ width	0.023	0.025	0.020	0.026
Acetabulum diameter	0.023	0.026	0.021	0.028

Precercarial stage: Sporocysts are long, white tubes of even diameter. Mature cercaria-filled sporocysts measure 0.22 mm in transverse section and may be four or five millimeters long.

Hosts: Cercaria obtained from numerous snails of *Stagnicola umbrosa* Say, and *Physella elliptica* Lea.

C. okobojensis is by far the most common strigeid larval form in the Okoboji region. It was obtained from snails of most of the lakes and sloughs where collections were made in each of the three years in which work was done.

Nine previously described forms have four pairs of post-acetabular penetration glands. They are: *C. Apatemon gracilis* (Rudolphi, 1819) Szidat, 1931, *C. helvetica* XXXI Dubois, 1929 (see also Wesenberg-Lund, 1934), *C. hamburgensis* Komeya, 1938, *C. longiremus* Wesenberg-Lund, 1934, *C. gracillima* Faust, 1917, *C. burti* H. M. Miller, 1923 (see also Cort and Brooks, 1928), *C. pseudoburti* Rankin, 1939a and *C. caperata* Olivier, 1942.

C. longiremus has no commissure and lacks distinct caudal bodies. *C. helvetica* XXXI has an anterior rather than a posterior commissure, *C. gracillima* has 22 caudal bodies, and *C. caperata* has no commissure and has two pairs of flame cells in the tail-stem. The forms most similar to *C. okobojensis* are *C. burti*, *C. pseudoburti*, *C. apatemon gracilis*, and *C. hamburgensis*.

The writer recognizes that dimensions are unreliable especially in view of the fact that they are taken in so many different ways and that even when they are taken in the same way such factors as cover-slip pressure, temperature and strength of killing agent, and elapsed time since the larvae were killed affect the measurements. In order to compare dimensions as favorably as possible, ten or more cercariae were measured alive and after having been killed by heat, by 10% hot formalin and when mounted in balsam, thus duplicating the methods of each of the previous authors of closely related forms. The results tabulated under *C. okobojensis* and the compari-

sons which follow are made between measurements given by the previous authors and those of the new species taken the same way. Measurements of *C. okobojensis* differs from Miller's measurements for *C. burti* in that the body is much smaller. Since this form tends to contract until it is almost circular and at other times to distend itself greatly, three sets of measurements can be taken which will vary considerably. When the body of *C. okobojensis* is extended, its length is about the same as that given by Miller, but the tail-stem length is then considerably less than the body instead of being greater. The measurements for *C. okobojensis* differ from those given by Cort for *C. burti* principally in the length of the tail-stem. The ratio of Cort's measurements for the length of the body and tail-stem is 122:165 while *C. okobojensis* was found to have the length of body and tail-stem nearly the same except when the body was in its contracted or extended condition. The width of the body of *C. burti* according to Cort's measurements indicates that it is a much larger form. Rankin's measurements of *C. pseudoburti* and Komiya's measurements of *C. hamburgensis* show a disproportionately larger tail-stem. Szidat's data for *C. Apatemon gracilis* indicate that it is a much larger form.

The present writer considers the difference in the position of the unpigmented "eyespot" as being significant. Both Cort and Rankin show these structures as being at the level of the fork of the esophagus. Szidat shows them midway between oral sucker and acetabulum, while Komiya shows complete absence of these structures. In *C. okobojensis* the "eyespot" are on a line with the front of the acetabulum or slightly posterior. They are also nearer the lateral margin of the worm than either Cort or Rankin show.

C. burti has four pairs of ducts leading from the penetration glands; *C. hamburgensis*, *C. pseudoburti* and *C. okobojensis* have two pairs. In the latter these are of fine caliber, but tend to become greatly distended in the anterior organ. Rankin shows and mentions ductal-distension, but the small nearly spherical bulbs that he shows were never seen in *C. okobojensis*.

The island of Cort in *C. okobojensis* is short and wide, while in *C. burti* and *C. pseudoburti* it is smaller and nearly circular and in *C. hamburgensis* and *C. Apatemon gracilis* it is absent altogether. The flame cell pattern in the five forms is the same.

C. okobojensis differs from *C. pseudoburti* in that the oral cap of spine covers less than half rather than three-fourths of the anterior organ. *C. pseudoburti* has two rows of irregularly spaced spines around the opening of the acetabulum. Cort says that the spines around the opening of the ventral sucker of *C. burti* are small and in two or three rows. *C. okobojensis* has four distinct and regular rows of fairly large spines.

Neither Miller, Cort, Rankin, Komiya, or Szidat shows well-marked individual cells in the oesophagus and ceca. These appear quite distinct in *C. okobojensis* and the last two cells of each cecum are septate.

Stunkard, Willey, and Rabinowitz, 1941, describe a cercaria having most features in common with *C. burti*, *C. pseudoburti*, the cercaria of *Apatemon gracilis*, and *C. okobojensis* and claim that the first three are the same, being the larval stage of *Apatemon gracilis*. Olivier, 1942, suggests that *C. helvetica* XXXI and *C. hamburgensis* may also be conspecific with the other three.

The form described by Stunkard, Willey, and Rabinowitz differs from *C. okobojensis* in that it has four instead of two ducts leading from the penetration glands,

a small, circular island of Cort, sixteen caudal bodies entirely in the tail-stem, and in that the body stem is distinctly shorter than the tail-stem (0.129 mm : 0.159 mm).

Giovannola, 1937, describes a form which he considers to be a variety of *C. burti* (*C. burti* var. *icnusae*). Apparently, the question is (1) whether the cercariae described by these various authors are a single cosmopolitan species whose small size is responsible for errors in description; (2) whether they are valid, but closely related species; or (3) whether they are distinct varieties of a single species. The present author believes that they represent at least several separate, but closely related species.

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LARVAL TREMATODES OF NORTHWEST IOWA. III. A NEW COLLARLESS ECHINOSTOME CERCARIA*

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The studies pursued by the author on the larval trematodes of the Lake Okoboji region in Iowa during the summers of 1940, 1941, 1942 have revealed a number of forms not previously described. In the present paper a new species of cercaria will be described which has the features of the echinostome group except that it lacks com-

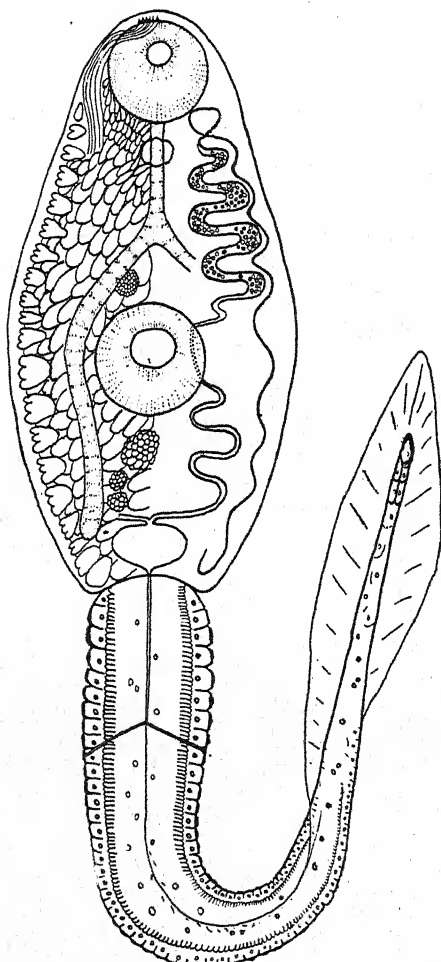


FIG. 1. *Cercaria ornatocauda* n. sp.

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The author is indebted to Professor T. C. Stephens of Morningside College, a member of the Iowa Lakeside Laboratory staff, for the identification of the snails mentioned in this study.

pletely the usual collar of spines and is therefore referred to as being echinostomoid. The taxonomic relationships of this form are discussed at some length because of certain problems involved.

Cercaria ornatocauda n. sp.

(Fig. 1)

Specific diagnosis: Echinostomoid cercaria belonging to Reflexae group. Body very large, seldom with straight sides; acetabulum larger than oral sucker, both small; tail longer than body, with dorso-ventral finfold and very ornate. Cystogenous glands abundant containing granular but not rod-like material. Penetration glands not distinguished, but ducts seen leading to six pairs of nozzle-like openings in front of oral sucker. Prepharynx nearly as long as pharynx; esophagus forks well above acetabulum; ceca extend to region of bladder. Excretory bladder bulbous; siphons proceed from common opening at top of bladder and pass over acetabulum beyond which they form from four to seven undulations on each side of body before making reverse loops near oral sucker to become recurrent tubules; recurrent tubules ciliated throughout most of length; siphons anterior to acetabulum comparatively narrow and loosely filled with small refractive granules; excretory canal forks in upper sixth of tail. Genital primordia consists of three masses of cells located eccentrically between bladder and acetabulum and one mass in front of acetabulum. No spines on body or tail.

Measurements: This large, muscular larva changes size and proportions almost constantly when alive, but under a given killing procedure measurements are fairly constant. Living cercariae range from 0.340×0.306 mm contracted to 1.180×0.080 mm extended. Average measurements in millimeters of cercariae when living, when killed by heat, and when killed in 10% hot formalin:

	Living, moderately distended	Killed in 10% hot formalin	Killed with heat
Body length	0.680	0.560	0.672
Body width	0.270	0.220	0.275
Tail length	0.730	0.745	0.720
Tail width	0.085	0.072	0.085
Oral sucker length	0.062	0.064	0.068
Oral sucker width	0.066	0.058	0.068
Acetabulum diameter	0.085	0.088	0.088
Prepharynx	0.026
Pharynx length	0.029
Pharynx width	0.026
Center acetabulum to posterior end of body	0.285

Precercarial stage: Cercariae produced in typical rediae with gut extending to locomotor organs and containing golden or orange-colored materials. Walls of rediae contain yellow pigment. Typical size of rediae 0.900×0.340 mm.

Host and Locality: Found in numerous specimens of *Stagnicola umbrosa* Say, *Stagnicola palustris elodes* Say, and *Helisoma trivolvis* Say from various lakes and sloughs in the Okoboji region.

Activity.—When free in water, *C. ornatocauda* swims actively with a characteristic wobbling movement. It flexes its body to form a ball from which the tail extends for locomotion. Under a coverslip it loops its tail to form a lateral 8, and maintains with it a characteristic quivering motion. Emergence may occur any time during the day and only a few are found swimming in a bottle at a time.

Other echinostomoid cercariae of the reflexae group are: *C. of Psilostomum reflexae* Cort, 1914, also described by Feldman, 1941; *C. of Himasthla militaris* Rudolphi, described by Van Beneden, 1861; *C. helvetica* XVII Dubois, 1928; *C. penthesilia* Faust, 1921; *C. sudanensis* I Archibald and Marshall, 1931; and *Psilostomum ondatrae* Price, 1931, described by Beaver, 1939. Of these, *C. ornatocauda* most resembles *C. of Psilostomum reflexae*. A significant difference lies in the sizes of the two larvae and the dimensions of their parts. Feldman does not give

the size of the body, but Cort, 1915, says that a well-extended specimen has a length of 0.46 mm and a width at the acetabulum of 0.135 mm. Moderately extended specimens of *C. ornatocauda* average 0.680×0.270 mm. Both Feldman and Cort give 0.046 mm as the diameter of the oral sucker and Cort gives 0.060 mm and Feldman 0.071 mm as the diameter of the acetabulum; these structures in *C. ornatocauda* are 0.062×0.066 mm and 0.088 mm respectively. The tail length of *C. ornatocauda* is likewise much greater. Feldman mentions 42 paired flame cells plus a median unpaired one. The cystogenous glands of *C. ornatocauda* are so very dense that no flame cells could be seen. Neither Feldman nor Cort mentions anything comparable to the ornate "lacy" tail that is a conspicuous feature of *C. ornatocauda*, nor do their drawings indicate that the tail is highly ornate.

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EXPERIMENTAL STUDIES ON THE FISH HOSTS OF
POSTHODIPLOSTOMUM MINIMUM
(TREMATODA: STRIGEIDA)

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On the basis of experimental and morphological studies *Cercaria multicellulata* Miller, 1923, and *Neascus vancleavei* (Agersborg, 1926) were considered by Hunter (1937) and Ferguson (1937) to represent developmental stages of *Posthodiplostomum minimum* (MacCallum, 1921). Furcocercous cercariae described as *C. multicellulata* by Miller were obtained from *Physa gyrina* collected near Urbana, Illinois. Cercariae identified as belonging to the same species were found by Ferguson to infect *P. integra* near Urbana, Illinois, and by Hunter to infect *P. gyrina* in the lower Hudson watershed, New York State. Encysted metacercariae of the *Neascus* type collected in the United States and Canada from 23 species of fish, distributed over six families, were reported by Hunter and Hunter (1940) to represent the last larval stage of *P. minimum*. Similarly, the metacercariae of *P. minimum* were reported from 18 species of fish by Ferguson. The adult stage of this strigeid was described as *Diplostomum minimum* from the great blue heron (*Ardea herodias*) by MacCallum (1921). Adults collected from several species of heron in the United States and Canada, and those obtained from two species of herons and chicks experimentally fed encysted metacercariae from minnows and sunfishes, were considered by Ferguson to represent the species of *P. minimum*.

In recent studies metacercariae believed to be those of *P. minimum* from the pumpkinseed sunfish (*Lepomis gibbosus*), collected at Princeton, N. J., and blackhead minnows (*Pimephales promelas*), obtained from E. W. Surber, U. S. Bureau of Fisheries, Leetown, W. Va., were fed to two groups of trematode-free black-crowned night herons (*Nycticorax nycticorax*). Separate lots of laboratory-reared *Physa acuta* and *P. heterostrophus* were exposed to miracidia hatching from the eggs of the adult strigeids developing in the herons from the metacercariae out of the minnow and sunfish hosts. Both species of snail became infected and released cercariae. Several dozen each of blackhead minnows and bluegill sunfish that had been held in the laboratory for several weeks were exposed to cercariae from *P. acuta* infected with the pumpkinseed line of strigeids. Similarly, several dozen each of blackheads and bluegills were exposed to cercariae from *P. acuta* infected with the blackhead line of strigeids. Bluegills exposed to cercariae of the sunfish line became infected and metacercariae were obtained after about 4 weeks, while blackheads similarly exposed were not infected. Bluegills exposed to cercariae of the blackhead line did not become infected, while blackhead minnows that were exposed became infected and fully developed metacercariae were obtained.

A small number of pumpkinseed sunfish, smallmouth bass, and largemouth bass after exposure to cercariae of the minnow line failed to become infected. However, after small numbers of the same three species were exposed to cercariae of the sun-

fish line, only the largemouth bass failed to become infected. From the pumpkinseeds and smallmouth bass, encysted metacercariae were obtained.

Klak (1939) stated that at the U. S. Bureau of Fisheries Station, Leetown, W. Va., blackhead minnows, blunt-nosed minnows, spot-tailed minnows, and attractive minnows were infected with encysted metacercariae of the *Neascus* type. When smallmouth bass, largemouth bass, and golden shiners were taken from the ponds in which the above mentioned minnows were found to be infected, they never contained any of the larval strigeids. These observations in the field confirm the above experimental evidence which revealed that the minnow line of cercariae did not infect either largemouth or smallmouth bass.

The blackhead minnows, bluegill sunfish, and pumpkinseeds used in the experiments all contained a small number of mature cysts of the *Neascus vancleavei* type when they were exposed to cercariae of either the sunfish or minnow line released from *P. acuta*. However, within the limited range of these experiments no immunity reaction seemed to be expressed when minnows and bluegills were exposed to cercariae of the minnow and sunfish line respectively.

Encysted metacercariae from several species of minnows (CYPRINIDAE) and sunfishes and smallmouth bass (CENTRARCHIDAE) were studied in detail by Ferguson (1937) with particular reference to size, morphology, and host relationship. At the same time similar studies were made of adults recovered both from naturally infected herons and from herons and chicks fed metacercariae from fish representing the above two families. Live and fixed metacercariae from the minnows, while similar in morphological details to metacercariae from members of the family CENTRARCHIDAE, were uniformly smaller. Likewise adults developing in experimentally infected birds from the encysted larval worms obtained from minnows were uniformly smaller than those adults developing from the centrarchid line of metacercariae. It was found that the type of fixative and its temperature were factors which determined to a certain extent the sizes of the preserved metacercariae and adults. Usually there was an overlapping of the size ranges of metacercariae from representatives of the two families of fish. The same was true for adults developing from these two lines of metacercariae. In minnows the encysted helminths were found largely in the liver, on the mesenteries, or free in the body cavity, while in the sunfish and smallmouth bass they mostly occurred in the liver and kidneys and on the heart. Since the minnows were much smaller in size than the sunfish it was believed that the size of the host in relation to the number of parasites infecting it, and the position in the host's body in which the larval worms developed, must have been responsible for the ultimate size attained by the mature metacercariae.

The experiments reported here indicate that there is a definite specificity with regard to the fish hosts which the two lines of cercariae can infect. This is particularly interesting since the same species of snail and bird will serve as host for the two lines. It was not possible to study critically the morphology of the cercariae representing the minnow and sunfish lines of this strigeid material. Differences of taxonomic value may eventually be demonstrated to exist among these cercariae. If this is not done it will have to be assumed that physiological factors determine the fish hosts which are infected. Only when these questions are settled can it be decided whether two species of the genus *Posthodiplostomum* are being dealt with or whether physiological varieties are represented under a single species concept.

Since MacCallum described the adult stage of *P. minimum* from a heron dying in the New York Zoological Park, it is impossible to know the source of the metacercariae infecting this bird. Miller described *C. multicellulata* from *Physa gyrina* collected in Urbana, Illinois, and Hunter in New York State was able to obtain infections in sunfish exposed to cercariae from *P. gyrina* which he identified as belonging to the same species. Ferguson, at Urbana, Illinois, exposed blunt-nosed minnows to cercariae identified as *C. multicellulata*, and these larval worms developed into metacercariae. Since both the sunfish and minnow lines of trematode material were able to infect *P. acuta* and *P. heterostrophus*, it is impossible to determine which line of cercariae Miller may have been dealing with. In view of the exposure experiments of Hunter with sunfish, and of Ferguson with minnows, both of whom believed they were working with *C. multicellulata*, apparently no significant difference was noted by these authors between their cercariae and the form described by Miller. However, further examination of these cercariae may reveal anatomical characteristics by which the lines may eventually be differentiated.

Neascus vancleavei was described by Agersborg from a species of minnow collected in Illinois and redescribed by Hughes (1928) from minnows collected in Illinois and sunfishes taken in Michigan. Hughes found that there was an overlapping of the size ranges of the metacercariae from the different fish hosts. Neither Hughes, Hunter, nor Ferguson detected any significant anatomical differences between metacercariae from the families CYPRINIDAE and CENTRARCHIDAE. If differences are eventually demonstrated between the metacercariae occurring in a bluegill sunfish and a blackhead minnow, those obtained from every species of fish in the two families mentioned will have to be carefully studied both morphologically and by infection experiments. If, as may be the case, there are at least two species or physiological varieties, it may be shown eventually that these forms are host specific as far as the family of the fish is concerned. Should two distinct species be proved to exist, only the metacercariae from minnows could be named *Neascus vancleavei*. In Hunter and Hunter, metacercariae considered to be those of *P. minimum* were reported from fish belonging to six families. The relation of all these metacercariae to one another would need to be investigated.

From the preceding discussion it becomes apparent that the life cycle relationships of MacCallum's adult worms and Miller's cercariae are unknown. The minnow origin of Agersborg's metacercariae is certain. In view of the uncertainties as to the specific status of the metacercariae now considered to be those of *P. minimum* it is evident that this confusion must be eliminated by morphological and experimental studies before the taxonomic status of the trematodes in question can be determined.

This confusion emphasizes once more that both morphological and life history experimental work must be done before it is possible to identify correctly stages in strigeid life cycles and evaluate specific concepts.

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A NEW MEDIUM FOR THE CULTIVATION OF *HISTOMONAS MELEAGRIDIS*

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Histomonas meleagridis, the protozoan parasite causing blackhead in turkeys, has been propagated in several different laboratory media since its discovery by E. E. Tyzzer in 1919.

Tyzzer (1) cultivated the organism in a medium composed of a buffered agar prepared with egg-albumin and covered with saline containing five per cent of sterile horse serum. Small quantities of rice starch and charcoal subsequently were added. This medium was reported to give good growth and to be somewhat less favorable to the growth of associated bacteria than the L.E.S. (Locke-egg-serum) medium of Boeck and Drbohlav (2). Bishop (3) cultivated the blackhead parasite in the inspissated horse serum slants of Dobell and Laidlaw (4). Cultures lasted five to seven days during a nine-month cultivation period. The serum slants were prepared by placing in culture tubes horse serum (sterilized by filtration) in suitable amounts and forming slants by heating to 80° C. The slants were covered with Ringer's solution containing four egg whites per liter. Previously Bishop had held cultures for an average period of three days at 37° C in a medium composed of inspissated whole-egg slants covered with a solution containing one part of horse serum in eight parts of Ringer's solution and with rice starch added before using. The short life of those cultures would appear to have resulted from the temperature employed rather than the type of medium, since the writer has held cultures of *Histomonas* on media of several kinds at 37° C but has never observed multiplication of the trophozoites at that temperature. Bishop reported a heavy overgrowth of bacteria as a result of using the liver medium of Cleveland and Sanders (5) and cultures died out after a few transplants. Bishop (3) also propagated the organism in a medium composed of one part of horse serum diluted 1:8 without a slope but with rice starch added before using (6). DeVolt and Davis (7) employed a L.E.S. medium with Locke's solution containing turkey serum and albumin or serum alone with subsequent additions of rice starch. Transfers were made every two or three days depending upon the relative numbers and kinds of associated bacteria in the cultures.

Experimental work designed to develop an improved medium for the cultivation of the blackhead parasite was undertaken by the writer in 1938. The necessity

of adding sterile supplements to the media of the older types which were never completely sterilized in the autoclave proved troublesome and a handicap to the cultivation and further study of the organism. In addition, fragmentation of the egg slant by associated bacteria produced a colloid and shortened the life of the cultures. As observations indicated the protozoon did not obtain sustenance directly from the slant, that part of the medium appeared unessential. Since the propagation of the parasite in pure culture (in the absence of associated bacteria) is the ultimate goal, a medium that could be sterilized largely en masse was sought.

A new and improved medium for the cultivation of *Histomonas meleagridis* was developed by eliminating the slant and sterilizing serum in Locke's solution after adjusting the pH value of the solution. Rice starch is sterilized separately and added before using. The formula of the medium follows:

NaCl	9.0 gm
CaCl	0.2 gm
KCl	0.4 gm
NaHCO ₃	0.2 gm
Glucose	2.0 gm
Fresh clear turkey serum	20.0 ml
Aqueous N/20 NaOH	20.0 ml
Distilled water	1000.0 ml

The medium is prepared by adding to Locke's solution two per cent of fresh turkey serum. Chicken serum is equally suitable and it is highly probable that the sera of other animals could be used as well in the preparation of this medium. Precipitation of the serum protein by heat in the autoclave is prevented by the addition of two per cent of aqueous N/20 NaOH as base. This adjusts the pH to approximately 9.0. Ten ml of the medium are placed in ordinary sized culture tubes (16 by 150 mm) and plugged with cotton. The medium is then autoclaved at 120° C for about 20 minutes. This heating period leaves the medium clear or produces not more than a barely perceptible turbidity which is well tolerated by the parasites. In selecting sera for the medium, care should be exercised to avoid the turbid sera commonly observed in fowls at the onset of the spring egg-production period. Such sera produce a stable colloid harmful to the parasites. The filtrate obtained after precipitating by heat clear serum in unadjusted Locke's solution has also supported cultures very well through several transplants.

Rice starch is sterilized in small tubes by dry heat at about 160° C for two hours. It is convenient to transfer a small quantity (one to two milligrams) of the sterile starch to culture tubes with a small scoop or spoon made from a piece of wire. A bowl is fashioned by flattening one end of the wire and turning up the edges. The bowl is then turned at right angles to the shaft which is fitted into the bottom of a small cork used to stopper the rice-starch tube. After heating the scoop in the flame of a bunsen burner the transfer is made after the mouth of both tubes have been flamed. A preliminary incubation period of 12 to 24 hours is sometimes used as a test of sterility of the completed medium.

If the parasite cultures are found to contain relatively large numbers of bacteria, interfering with the functions of the protozoon, it is well to eliminate glucose from the medium and reduce the concentration of serum to one per cent as both components are favorable to the overgrowth of bacteria. That certain bacteria exist with cultures of the blackhead parasite in some degree of symbiosis is shown by the

failure of *Histomonas meleagridis* to survive in cultures partially freed from bacteria by sedimentation. At the same time, trophozoites sedimented into the usual suspension of associated bacteria reproduced as before.

Cultures of the blackhead parasite have been propagated in the above medium for several periods of a year or more. Transfers ordinarily are made twice a week and viable organisms can be found in the medium after one or two weeks. The parasite has been isolated repeatedly from the cecal contents and on several occasions from the liver. In making liver cultures the surface of the organ is seared and a wire loop used to transfer three or four loopfuls of blood and hepatic tissue to each of a dozen or more culture tubes previously tested for sterility. Not more than one or two positive tubes have been found per dozen inoculated. The parasites have in every instance been found associated with mixed bacterial cultures.

The new medium offers the advantage of less frequent transfer, sterilization largely en masse, and easy preservation. A medium of this type lends itself more readily to investigations of the relationship between the protozoa and bacteria associated with them.

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OBSERVATIONS ON THE USE OF SEA WATER IN THE CONTROL OF *ANOPHELES ALBIMANUS* WIED.

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In Puerto Rico, *Anopheles albimanus* breeds prolifically in certain coastal lagoons having varying concentrations of sea water. The degree of salinity is altered by tides, evaporation and rainfall. These factors operate to create favorable or unfavorable conditions for mosquito breeding according to the tolerance of the species. It has been recognized that *A. albimanus* will not tolerate high concentrations of sea water and in certain instances this mosquito has been controlled by the introduction of sea water into land locked coastal lagoons (Washburn, 1933, and Tullock, 1937). The investigation here recorded was made in order to obtain more precise information on the limiting concentration of sea water required for the control of this species and the means by which such a concentration can be maintained in certain natural breeding places.

It was found convenient to express the relative salinity in terms of per cent of sea water as determined by a hydrometer. Either a sodium chloride hydrometer or a urinometer may be calibrated from known dilutions of sea water. Eighty degrees Fahrenheit was taken as a convenient temperature and calibrations were made at five per cent intervals.

LABORATORY OBSERVATIONS

The per cent hatchability of eggs deposited by isolated females in known dilutions of sea water was finally chosen as the principal criterion for determining the limiting effect of sea water on the development of *A. albimanus*. The rearing of larvae introduced so many uncontrolled factors that data from this source proved to be less useful. All the ovipositing females were collected in nature within a radius of two miles. Specimens from each collection were divided so that examples were distributed to each of the test concentrations of sea water. The collections were made over a period of three months beginning in October and continuing through December.

Gravid or engorged females were isolated in shell vials approximately 25 mm in diameter and 100 mm long, containing about 25 mm of diluted sea water. The vials were lined in the lower two thirds with absorbent paper and stoppered with cotton. Dilutions of 25, 50, 75, and 80 per cent sea water were used. Deposited eggs were left in the vials for 7 days at approximately 80° F. They were then removed from the vial on the absorbent paper lining and the total number of eggs and

TABLE 1.—*Deposition and hatching of A. albimanus eggs in various dilutions of sea water*

Per cent sea water	No. females segregated	Per cent ovipositing	Per cent batches infertile	Av. per cent hatching per batch	Range per cent hatching	Av. No. eggs per batch	Range No. eggs per batch
Distilled water	120	33.3	20	65.5	0-100	113	16-211
25	103	31.1	3	33.1	0-76	111	10-222
50	130	15.4	30	7.7	0-32	97	13-194
75	74	20.3	73	6.6	0-45	104	17-174
80	171	10.5	89	0.33	0-5	86	27-184

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the number hatched determined by microscopic examination. The data are presented in Table 1.

The average per cent hatching per batch decreased with increasing concentrations of sea water. The average for distilled water was 65.5 per cent, for 25 per cent sea water 33.1, for 50 per cent sea water 7.7, for 75 per cent sea water 6.6 and for 80 per cent sea water 0.33 per cent. Twenty per cent of the batches deposited in distilled water were abnormal in that the chorion did not turn black after deposition. These eggs do not hatch. This phenomenon is observed quite frequently in eggs deposited in the laboratory. The relative number of such batches increased in the higher concentrations of sea water until in 80 per cent sea water only 11 per cent of the batches attained the black appearance of normal eggs and of these but a small percentage hatched (Table 1). The average number of eggs per batch did not appear to be affected significantly by the salt concentration but the number of mosquitoes actually ovipositing decreased in the higher concentrations of sea water. Under these experimental conditions one may expect about one third of the gravid females to oviposit on distilled water but only about ten per cent on 80 per cent sea water.

In two instances eggs from a single batch deposited on distilled water were divided after 5–15 hours and the fractions distributed on 66, 75, 80 and 100 per cent sea water. Under these circumstances the lethal concentration of sea water appeared to be between 66 and 80 per cent (Table 2).

TABLE 2.—The effect of various concentrations of sea water on the hatching of eggs deposited by the same female. The eggs were deposited on distilled water and allowed to remain 5–15 hours before removal to diluted sea water

Specimen		Distilled water	66 2/3% sea water	75% sea water	80% sea water	100% sea water
I	No. of eggs	21	21	X	21	20
	Per cent hatching	57	28	X	0	0
II	No. of eggs	48	X	44	46	X
	Per cent hatching	77	X	0	0	X

Table 3 gives the data obtained from laboratory rearing of *A. albimanus* in diluted sea water and in tap water. Dried yeast was supplied as food. The eggs in each case were deposited at one time by a segregated female on the concentration of

TABLE 3.—Rearing of *A. albimanus* in diluted sea water

Per cent sea water	No. of eggs	Per cent hatching	Per cent reaching adult stage	No. of days to complete cycle
0	61	67	33.0	14
33	123	80	21.0	12
33	163	45	6.0	12
75	131	15	2.0	16
75	115	45	0.9	17

sea water indicated. The salt concentration was maintained approximately the same during the larval and pupal stages by adding distilled water to compensate for evaporation. The uncontrolled factors introduced in rearing the larvae make the data of limited value. However, it does indicate that this species is capable of passing through all its aquatic stages in concentrations of sea water as high as 75 per cent.

The survival of 1st instar larvae collected from a brackish water breeding place was also studied when they were subjected to higher concentrations of sea water. Sea water was added to the water taken from the natural breeding place until estimated concentrations of 50, 75, 80 and 90 per cent were reached as determined by a hydrometer. The water from the natural breeding place was estimated to be the equivalent of about 25 per cent sea water. Yeast was utilized as food. The data are presented in Table 4. Twelve per cent of those in 80 per cent sea water attained the adult stage but none of those in 90 per cent sea water survived beyond the second instar.

TABLE 4.—*Survival of A. albimanus larvae collected in 25% sea water when subjected to higher concentrations*

Per cent sea water	No. of 1st instar larvae	Per cent reaching pupal stage	Per cent reaching adult stage
25	17	82	65
50	17	65	6
75	17	35	18
80	16	19	12
90	15	0	0

FIELD OBSERVATIONS

An opportunity for observing the effect of gradually increasing salinity on *A. albimanus* production in a natural breeding place was afforded by the introduction of sea water into a coastal lagoon where this species was breeding in abundance. Table 5 gives the rate of increase in salinity as determined by almost daily hydrometer readings. Larval densities were determined by counting the number of larvae per 40 dips in a selected area of approximately 100 square feet. The salinity samples were taken from the same location. The daily collections of larvae classified according to instar are recorded in Table 5. All larvae were returned to the

TABLE 5.—*The effect of increasing sea water concentration on the production of A. albimanus in a natural breeding place*

Date	Per cent sea water	Larval instars					<i>A. albimanus</i> identified	Remarks
		1	2	3	4	Pupae		
Oct. 10	15
11	20
12	25	50	3	5	10	0	68
13	30
14	35
16	50	Oil applied
19	65	18	0	0	0	0
20	65	4	13	0	0	0	..	Some newly hatched
21	75	0	11	2	0	0
22	65	1	7	16	1	0
23	70	0	0	2	1	0	1
24	75	0	0	1	3	1	2
26	75	0	0	0	2	0	2
27	85	0	0	1	4	0	3
29	80	0	0	0	2	2	4
30	90	0	0	0	1	0	2	1 pupal skin
31	85	0	0	0	0	1	1
Nov. 2	90	0	0	0	1	1	2
4	85	0	0	0	0	0	..	1 <i>A. grābhami</i>
5	5	4.5 in. rain
6	15	0	0	0	0	0
8	20	0	1	0	0	0	1
10	40	1	1	0	0	0	2
12	65	0	0	0	0	0
14	85	0	0	0	0	0
16	90	0	0	0	0	0
18	85	0	0	0	0	0

breeding place except the collections of October 12th and 19th. Identification indicated that the larvae were almost 100 per cent *A. albimanus*. It will be observed from Table 5 that practically no 1st instar larvae were encountered after October 20. This would indicate either that oviposition ceased about October 15 when the relative salinity was about 50 per cent, or eggs deposited after that time failed to hatch. However, the larvae already present continued to develop and an observable number reached maturity even though the relative salinity had by that time exceeded 80 per cent. These observations agree reasonably well with those made in the laboratory. Continued dipping throughout the marginal area of the lagoon indicated that the production of *A. albimanus* had been virtually eliminated.

Prior to the establishment of a sea connection, collections made over a period of six weeks indicated the presence of *A. albimanus* in abundance with the relative salinity remaining between 15 and 25 per cent sea water. Concurrent samplings in an adjacent lagoon separated from the first by a roadway fill and communicating with the sea by a small channel were entirely negative. The salinity here varied between 70 and 80 per cent.

It appears from the foregoing observations that active breeding of *A. albimanus* ceases in water having a salinity much exceeding that of 50 per cent sea water. The continued presence of larvae probably indicates that the salinity has recently been much lower or the larvae have been washed in from some fresh water habitat.

MAINTAINING THE DESIRED SALINITY IN NATURAL BREEDING PLACES

In practice, it is desirable to maintain the salinity high enough to allow an adequate margin of safety above the point where active breeding ceases. Seventy-five per cent sea water is believed to furnish this margin. Tidal action in the lagoon was found to be the most potent factor in distributing the salt to remote shallow areas where mosquito breeding is most likely to occur. Daily tidal fluctuations of 5 or 6 inches were obtained in the lagoon under observation. This resulted in a rapid increase in salinity until it stabilized between 80 and 90 per cent (Table 5). Four and one half inches of rain on November 5th brought it as low as 5 per cent but within a week it had returned to the former stabilization level.

The lagoon under observation had an area of approximately 400 acres and an average depth of about 1.5 feet. It was divided along its long axis into two nearly equal parts by roadway fills. An opening to the sea 55 feet wide and another 30 feet wide through the roadway fills proved to be adequate to produce the required tidal action and thereby maintain the necessary salinity. The average tidal range in this locality is 0.8 foot. The distance from the sea outlet to the most remote part of the lagoon was about two thirds of a mile.

OTHER ASPECTS OF TIDAL ACTION AND INCREASED SALINITY

No doubt, the mechanical action of the daily water level fluctuation exerted beneficial effects independent of salinity by stranding flotsam and exposing the larvae to their natural enemies through disturbance of the surface cover. Top feeding minnows were numerous. The beneficial influence of water level fluctuation in the control of *A. quadrimaculatus* in impounded water has been reported by Hinman (1938).

The increased salinity and tidal action produced marked changes in the flora. No detailed study was made of this but it was observed that a very troublesome plant, widgeon grass (probably *Ruppia maritima*) stopped spreading and was largely eliminated. It grows submerged in shallow brackish water forming dense mats at the surface. This makes a particularly favorable habitat for *A. albimanus*.

SUMMARY

1. *A. albimanus* breeds abundantly in certain brackish coastal lagoons in Puerto Rico.
2. Breeding of this species can be eliminated by increasing the relative salinity to about 75 per cent sea water.
3. This degree of salinity can best be maintained in marginal areas of lagoons by tidal action produced by an adequate sea connection.
4. A technique is described by which the tolerance of a mosquito for different salt concentrations can be tested by observing the effect on the hatching of the ova.

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RESEARCH NOTES

NOTES ON SOME PARASITES OF THE MINK IN SOUTHERN MICHIGAN

As a part of a more complete study dealing with the mink (*Mustela vison mink*) the writer autopsied 158 mink carcasses which were obtained from southern Michigan fur-buyers during the winters of 1940 and 1941. In addition to measurements taken on the carcasses, an effort was made to determine the incidence of some of the more important macroscopic parasites.

From a wildlife management standpoint it is important to know the incidence of parasites in various animals in order to evaluate the rôle which they play in regulating animal populations. There is still relatively little known concerning some of the parasites infecting our wild animals and the effects which they produce. Until recent years the approach has been largely taxonomic and morphologic, but if cyclic phenomena and trends of animal populations are to be correctly interpreted, the proportion of the population infected and the severity of the infections from year to year must be known. Although it is not to be expected that parasites infecting animals in the wild state can be controlled to any great extent, a knowledge of their distribution, incidence, life cycles, and modes of transmission may serve to prevent them from becoming a menace to ranch-bred animals.

The mink spends a considerable portion of its time in or near water and hence many aquatic animals eaten by mink, such as crustaceans, frogs, and fish, act as intermediate hosts for the large assortment of flukes and roundworms which habitually infect it. In common with the muskrat, the mink is often referred to as a virtual "museum of parasites."

Thirty-two carcasses were autopsied in 1940 and 126 in 1941, and the incidence of the most frequently encountered parasites is presented in Table 1. No significant differences in the two years were shown when the chi square test was used.

TABLE 1.—The incidence of some helminthic parasites found in mink taken during the trapping seasons of 1940 and 1941

Species	Year	Number infected	Incidence (%)	Average incidence two years (%)
<i>Skrjabinogylus nasicola</i>	1940	32	100	94
	1941	116	92	
<i>Filaroides bronchialis</i>	1940	3	9	19
	1941	27	21	
<i>Paragonimus kellicotti</i>	1940	2	6	9
	1941	13	10	
<i>Physaloptera</i> sp.	1940	0	0	4
	1941	6	5	
<i>Diocotophyme renale</i>	1940	1	3	3
	1941	3	2	

The sinus worm, *Skrjabinogylus nasicola*, appears to be the most common nematode parasite of the mink. The worms which occur in the frontal sinuses of the animal are frequently overlooked. In heavy infections the frontal sinuses were inflated and discolored by the mass of worms within. A similar condition commonly noted in skunks is caused by the related species, *S. chitwoodorum*. In one mink 45 of these worms were recovered from the two sinuses although the usual number found was about a dozen individuals. In a few instances the worms had apparently bored out through the wall of the sinus into the overlying muscle.

Lungworms, *Filaroides bronchialis*, are perhaps next in importance to the sinus worm. They occur as small, compact knots of closely intertwined worms which are usually situated along the trachea and bronchi and on the surface of the pulmonary vein. A few of these knots of worms were occasionally noted on the surface of the lung, and as many as six were found in the lungs of one mink.

The lungfluke, *Paragonimus kellicotti*, which is an occasional parasite in the lungs of cats, dogs, and pigs, finds its natural definitive host in the mink. Various species of crayfish belonging to the genus *Cambarus* constitute the second intermediate host while the snail, *Pomatiopsis lapidaria*, is the first (Ameel, 1934, Am. J. Hyg. 19: 279-317). An incidence of 8.09 per cent is reported by Wallace (1931, Am. Fur Breeder, 3: 24-25) from 84 carcasses received from fur farms in Minnesota. This compares closely with the 9 per cent incidence of infection which the author found in wild mink. Four cysts were found in the lungs of one mink with as many as six flukes to a cyst, but usually only a single cyst was found.

Larval and adult *Physaloptera* sp. occurred in the stomachs of six minks, one of them containing ten. In an animal heavily infected with this parasite the wall of the stomach was very much thickened.

The giant kidney worm, *Diocotphyne renale*, was found in only four of the 158 mink which were autopsied. Though not occurring as frequently as other parasites this worm is probably more destructive in the wild because of the total damage which it inflicts on the infected organ. In one of the parasitized animals a compensatory hypertrophy of the uninfected kidney was noticeable. A large calculus was present in the kidney of another.

Several other parasites were recorded from the minks which were examined, but they were probably of little importance. *Coccidia*, *Isospora bigemina*, and the eggs of *Capillaria* sp. were frequently noted in fecal samples together with larvae and adults. An adult female capillarid was recovered from the stomach of one mink.

Acknowledgment is made to Mr. J. T. Lucker of the Zoological Division, Bureau of Animal Industry for aid in identifying some of the parasites and for confirming the author's identifications, and to Mr. B. T. Ostenson of the Zoology Department, Michigan State College for general supervision.—JOHN A. SEALANDER.

TWO FLATWORMS FROM THE OYSTER-DRILLING SNAIL. *THAIS FLORIDANA* HAYSÆ CLENCH

During the course of investigations on the salinity tolerance of the Gulf Coast oyster-drilling snail, *Thais floridana*, made during the spring of 1942, the author had occasion to observe two associated flatworms. Polyclad turbellarians were obtained frequently from Barataria Bay, La. snails, *T. floridana haysæ* Clench, and occasionally from those of Santa Rosa Sound, Fla., *T. floridana floridana* Conrad. They emerged from the mantle cavities of their hosts after the snails had been immersed in water of low salinity, 10 p.p.1000 and below, for a few days. Some Barataria Bay specimens have been identified by Dr. Libbie Hyman as *Hoploplana inquilina* Wheeler, 1894. In a communication of May 28, 1942, Dr. Hyman stated that two of the specimens exceed the maximum size recorded for the same species from Woods Hole, Mass. (Hyman, L. H., 1940, Proc. U. S. Nat. Mus. 89: 449-495). Pearse and Wharton (1938, Ecol. Monographs 8: 605-655) have reported "*H. thaisana*," which may or may not be the same as *H. inquilina*, from *T. floridana floridana* Conrad obtained in Apalachicola Bay, Fla. Stauber (1941, J. Parasitol. 27: 541-542) discovered *Hoploplana inquilina thaisana* Pearse, 1938, in two species of snails associated with the oyster beds of Delaware Bay. He, too, observed that the worm abandoned its host at low salinities. Since the Delaware Bay and Florida specimens are both smaller than those of Woods Hole it appears that the largest of all are to be obtained in the Barataria Bay region. It is interesting to note that *Thais* of this region is also of extremely large size. Barataria Bay snails were also occasionally infected with trematode cercariae, which emerged when the snails were immersed in water of relatively high salinity, 25 p.p.1000 down to 14 p.p.1000. Specimens were submitted to Dr. H. W. Stunkard and identified by him as *Parorchis acanthus* (Stunkard, H. W. and Cable, R. M., 1932, Biol. Bull. 62: 328).

In so far as the author is aware neither *Hoploplana inquilina* nor *Parorchis acanthus* has previously been reported from the Barataria Bay region, west of the Mississippi River, nor from the host with which they are there associated. It is of some interest that they appear to have been enabled to cross the natural barrier of fresh water with the aid of transplantation methods used in cultivating Louisiana oysters (McConnell, J. N. and Kavanagh, L. D., 1941, Bull. No. 1, State of Louisiana, Department of Conservation).—VICTOR SCHECHTER, City College of New York, and the United States Fish & Wildlife Service Laboratory, Pensacola, Florida.

THE SELECTIVE ACTION OF SULFAGUANIDINE ON AVIAN COCCIDIA

Previous reports have indicated that sulfanilamide and sulfapyridine were inhibitory for those species of chicken coccidia which completed their entire development in the small intestine (Levine, 1939, Cornell Vet. 29: 309-320; 1940, J. Parasitol. 26: 233-235). In these studies neither *Eimeria tenella* nor *E. necatrix*, the oöcysts of which are formed in the ceca, were adversely affected. Investigation of other sulfonamide compounds led to the discovery that sulfaguanidine, when fed to chickens in concentrations of 0.5% of the ration, apparently suppressed completely all the species with the exception of those mentioned. The severity of infections with those two species was markedly reduced by increasing the concentration of the drug to 1.0% and 1.5% respectively (Levine, 1941, Cornell Vet. 31: 107-112; 1942, Cornell Vet. 32: 430-439).

Since the quantitative methods used in these studies did not permit recognition of infections when fewer than 40 oöcysts per gram of feces were present, the following critical experiments

were done to determine whether infections could be completely prevented by the feeding of sulfaguanidine. Two chickens, reared coccidia-free, were dosed with a mixture of pure "cultures" of sporulated oöcysts of all the species affecting chickens; *E. acervulina*, *E. praecox*, *E. mitis*, *E. hagani*, *E. brunetti*, *E. maxima*, *E. tenella* and *E. necatrix*. One of the birds had been fed mash containing sulfaguanidine in the proportion of 0.5% by weight for 48 hours previous to the infective dosing. This bird was kept on the medicated feed for the duration of the experiment. The second bird received non-treated mash and served as a control. Daily fecal examinations (sugar flotation) were made.

The control bird became infected with all of the species as evidenced by the appearance of their oöcysts in the feces, those of *E. praecox* and *E. acervulina* on the fourth day and others later. Post-mortem examination on the eighth day revealed the presence of typical lesions of *E. tenella* and *E. necatrix*. Oöcysts were not found in the feces of the treated bird until the seventh day at which time oöcysts similar to those of *E. tenella* and *E. necatrix* were observed. A bloody fecal discharge had been noted from this bird five days after the feeding of oöcysts indicating that infection with *E. tenella* had taken place. To establish the identity of these oöcysts, about ten grams of feces from the treated bird were collected on the eighth day and incubated for three days in shallow layers of 1½% potassium dichromate solution at 30° C to effect sporulation. If species other than *E. tenella* and *E. necatrix* were present (but missed on fecal examination), their oöcysts would surely be in this fecal sample since their life cycles are shorter. The incubated feces were fed to two coccidia-free chickens and again daily fecal examinations were done. It is generally agreed that this is the most delicate method of detecting the presence of oöcysts which may have been missed in routine fecal examinations. When no oöcysts were found up to and including the sixth day after dosing, one of the birds was destroyed for examination. Gross lesions typical of infections with *E. tenella* and *E. necatrix* were observed. Significantly enough, scrapings of mucosa from the small intestine when examined microscopically, were not only free from oöcysts but also from the intermediate stages of all of the other species. Examinations of feces from the remaining bird showed the presence of oöcysts on the seventh day and once more infections with only *E. tenella* and *E. necatrix* were confirmed. This experiment was twice repeated with identical results.

It is apparent that when sporulated oöcysts of *E. acervulina*, *E. praecox*, *E. mitis*, *E. hagani*, *E. brunetti*, and *E. maxima* are ingested by chickens being fed sulfaguanidine in concentrations of 0.5% of the ration, infection is completely prevented. The absence of intermediate stages from the intestinal mucosa indicates that the destruction of the inocula may have occurred even before invasion of the epithelium was effected. The fact that *E. tenella* and *E. necatrix* survive, makes this method valuable for the isolation of these species.—P. P. LEVINE, *New York State Veterinary College, Ithaca, N. Y.*

A NEW LOCALITY FOR *TRYPANOSOMA VESPERTILIONIS* (= *T. CRUZI* ?) IN BATS IN THE UNITED STATES

Dias (1937, Tr. Roy. Soc. Trop. Med. & Hyg. 31: 260) reported a trypanosome morphologically similar to *Trypanosoma* (*Schizotrypanum*) *cruzi* from the Pacific pallid bat, *Antrozous pallidus pacificus*, from Pinole, Contra Costa county, California. The writer believes that the extensive experience of Dr. Dias with bat trypanosomes justifies his identification. Since no one has yet proven conclusively that this organism is *cruzi* by demonstration of its host relationships, the writer believes it is more accurate to call it *Trypanosoma vespertilionis*.

On July 13, 1941, one of my former students, Robert O. Ingman, made two blood smears from an adult female *Antrozous pallidus pacificus* from seven miles west of Plymouth, Amador county, California. Examination of both films reveals two trypanosomes on one slide identical with *Trypanosoma vespertilionis* as illustrated by Wood (1941, Am. J. Hyg. 34: 4). This observation emphasizes the importance of investigating thoroughly the great central valley of California, especially the Sierra Nevada foothill areas, for demonstration of the causative agent of Chagas' disease in mammals and blood-sucking arthropods. Wood (1942, Los Angeles City Coll. Prog. Bull. 1: 31-34) has already reported the existence of *Trypanosoma cruzi* in *Triatoma protracta* from human habitations at Trimmer Springs, Fresno county, California.—SHERWIN F. WOOD, *Department of Life Sciences, Los Angeles City College, Los Angeles, California.*



W. H. Lewis

IN MEMORIAM

WINFIELD CAREY SWEET (1891-1942)

On May 20, 1942, Dr. Winfield Carey Sweet died suddenly of a heart attack in Cochabamba, Bolivia. He was born in La Crosse, Wisconsin, on December 21, 1891. Until he was sixteen he lived in China, where his parents were missionaries. His collegiate training was in Colgate University from which he graduated in 1913, and he received his medical degree from Rush Medical College in 1917. Later he studied at the Johns Hopkins School of Hygiene and Public Health, receiving the M.P.H. degree in 1927 and the Dr.P.H. in 1931.

Dr. Sweet was a medical officer in the U. S. Army from July, 1917, to June, 1919, spending nine months in Italy. After the war he served for nine months with the American Red Cross in Siberia and then went to the Christian Hospital, Shoahsing, Chekiang Province, China, where he took the place of a staff member on leave.

Dr. Sweet became a member of the field staff of the International Health Division of the Rockefeller Foundation on November 10, 1921. His first assignment was to Australia where he assisted Dr. W. A. Sawyer in developing a hookworm campaign, which later broadened out to include surveys on malaria and filariasis. This gave him the interest in parasitology which continued throughout the rest of his career. His next post was in Ceylon, where he directed the hookworm and rural sanitation program until the end of 1925. Then he returned for a year and a half study leave at the Johns Hopkins School of Hygiene and Public Health. During this period, he spent four months in Panama on hookworm investigations with Dr. W. W. Cort. In June, 1927, he went to India where he was stationed for the next twelve and one-half years, except for another year of study leave in Baltimore. Until 1938, his headquarters were in Bangalore in the State of Mysore. His work in India began with a hookworm and malarial survey. Later he served as a consultant to the government on public health. While in Bangalore he also maintained supervision over the health programs in Madras and Travancore. During his last eighteen months in India, he was stationed first in Delhi and then in Calcutta.

In February, 1940, Dr. Sweet was transferred to China to take charge of a program of investigations of malaria on the Burma Road. In the summer of 1941 he returned to the United States on leave expecting to go back to China, but was prevented by the outbreak of the war with Japan. Instead, he was given an assignment in Bolivia and left for South America on April 2, 1942. After a short stay in Buenos Aires he started his work of organizing the new hookworm and malaria programs for the Bolivian Health Department on May 6, only two weeks before his death.

Dr. Sweet was fundamentally interested in research and he found time in spite of extensive administrative duties not only to carry on investigations but to publish his results. Of his forty-four papers, all but three are in parasitology. They are chiefly concerned with problems of malaria and hookworm disease in the countries where he worked. In his researches on malaria he studied not only the distribution, epidemiology and control of the disease, but also the mosquito vectors. He also made important contributions on the epidemiology and control of hookworm disease

in Australia and India, and the researches which he and Dr. V. N. Moorthy made on the guinea worm are fundamental contributions on the biology and epidemiology of this parasite. All his work showed the happy combination of a very practical public health viewpoint with a sound biological approach.

Dr. Sweet well exemplified the spirit of the organization that he represented, combining the missionary with the scientific spirit. He was modest and unassuming, while forceful and effective in his work. He was truly a citizen of the world and served as an ambassador of good will by bringing the best of American public health progress to the peoples with whom he worked. His untimely death at the height of his career is a great loss since men of his type were never more needed than at the present time. He will be missed by his many friends all over the world.—W. W. CORT, *Department of Parasitology, Johns Hopkins School of Hygiene and Public Health.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Notice of Postponement of Annual Meeting of the Society

On July 15, 1943, Mr. Joseph B. Eastman, Director of the Office of Defense Transportation of the Office of Emergency Management, Washington, D. C., issued a statement with respect to the effect that conventions and group meetings have upon the nation's transportation facilities and urged that whenever possible such gatherings be postponed or cancelled. His statement follows, in part:

"... Conventions, even small conventions, produce concentrations of passenger traffic which severely impair the ability of the railroads and bus lines to provide adequate passenger service for military and essential war business travel. The passenger transportation problem is now so serious that I feel obliged to renew and reiterate, in the strongest terms, my request for cancellation of all such gatherings. . . .

"Each organization must make its own independent decision. The Office of Defense Transportation cannot pass upon the essentiality of any proposed meeting. It can only emphasize the serious burdens which convention and group-meeting travel imposes on transportation and request voluntary action by those who have it in their power to eliminate such travel. . . ."

In accordance with Mr. Eastman's request, the Council of the American Society of Parasitologists has voted to postpone the next annual meeting of the Society until travel restrictions are lifted.

JAMES T. CULBERTSON,
Secretary.

August 18, 1943.

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ESTIMATION OF HISTAMINE IN THE BLOOD AND OTHER TISSUES OF RATS AND GUINEA PIGS INFECTED WITH *TRICHINELLA SPIRALIS*¹

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Although the life cycle of *Trichinella spiralis* (Owen) is well understood and the histopathology and immunology of trichinosis have been extensively investigated, little is known concerning the toxicology of the infection. Since one of the products of tissue destruction is believed to be histamine, and the acute symptoms of trichinosis appear at the height of tissue invasion and presumably tissue destruction, an investigation has been made concerning changes in the histamine content of the blood and certain other tissues of albino rats and guinea pigs experimentally infected with *T. spiralis*.

Several investigators have suggested the possibility that the destruction of tissue and irritation caused by the migrating larvae of *T. spiralis* might result in the production of toxic materials and chemical changes in the blood. The first attempt to detect such poisons and determine their chemical nature was made by Flury (1913), who reproduced the symptoms of trichinosis by the subcutaneous injections of aqueous and alcoholic extracts of heavily infected muscles. He attributed the effects to no one particular substance but rather to a group of purine bases and creatine derivatives, such as methyl guanidine. Harwood, Spindler, Cross and Cutler (1937) using the method of Minot and Dodd (1933), reported an increase in guanidine in rabbits infected experimentally with *T. spiralis*. While increased guanidine has been reported in several diseases, its significance has been questioned (Sollman, 1942).

Histamine has a more pronounced pharmacological action than does guanidine and may possibly be a significant toxic agent in trichinosis. Increases of this substance in the blood and other tissues have been reported in a number of conditions including severe burns (Rosenthal, 1937), anaphylactic shock (Code, 1939), traumatic shock (Dale and Laidlaw, 1919), and adrenalectomy (Rose and Browne, 1941).

PROCEDURE

The strain of *Trichinella spiralis* used in the present study was carried in albino rats to provide larvae for experimental infections. Rats were fed ground infected

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¹ Based upon a thesis submitted to the Faculty of Purdue University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, May 3, 1942.

² The writer wishes to express his appreciation to Professor R. M. Cable for his direction and encouragement in the course of the present study.

muscle tissue while guinea pigs were infected with larvae from artificial digests, administered by stomach tube. Rats were given doses of from 600 to 10,000 larvae, and guinea pigs 1,000 to 3,000. After considerable experimentation, 6,000 larvae per rat and 3,000 per guinea pig were found to be satisfactory for producing symptoms of trichinosis. Whenever possible, litter mates were employed in each series. Symptoms were more uniform in guinea pigs than in rats, supporting Roth's (1939) opinion that the guinea pig is the better animal for experimental trichinosis. Since animals are sometimes resistant to infections with *T. spiralis*, the diaphragms of all experimental rats and guinea pigs were examined microscopically before proceeding with assays for histamine. Most of the histamine determinations were made on rats 16 to 18 days after larvae were administered, and on guinea pigs, 23 to 26 days.

Histamine in the blood was determined by the method of Barsoum and Gaddum (1935) as modified by Code (1937); other tissues, including lung, liver, intestine, skeletal muscle and kidney, were extracted according to the method of Best and McHenry (1930) as modified by Rose and Browne (1941). All assays were carried out on segments of isolated guinea pig ileum removed from animals that had been starved 24 hours. Refrigerated segments, as used by Minard (1941), were unsatisfactory because they were not sufficiently sensitive to the concentrations of histamine involved. The test strip was exposed to a standard histamine hydrochloride until a maximum contraction was obtained (never more than 30 seconds), then washed for 30 seconds in Tyrode's solution, permitted to rest 30 seconds, exposed to a standard or extract until a maximum contraction was obtained, then washed with saline, and the assay repeated in the same sequence. This order was rigorously maintained during the assay to obtain consistent contractions of the intestinal segment. Several determinations were made on each extract before it was discarded.

Rosenthal's (1937) method utilizing small amounts of whole blood was tried with a number of infected animals in an attempt to follow changes in histamine content as the infection advanced.

Differential white cell counts were made on a number of animals, using Giemsa stained smears. No attempt was made to differentiate the various types of lymphocytes.

RESULTS

Attempts to Estimate Blood Histamine Content by the Whole Blood Method of Rosenthal

Attempts were made to determine the blood histamine in 69 rats and 18 guinea pigs by assaying 2-4 per cent suspensions of whole blood in saline on the isolated ileum of the guinea pig. It was found that slight contractions of the intestinal segment resulted but consistent quantitative determinations could not be obtained and there was a rapid loss of activity. There was some indication that a substance or substances, which caused the contraction of the ileum, did increase in infected animals. The method gave such erratic results, however, that its use was discontinued.

Estimation of Blood Histamine by Code's Modification of the Extraction Method of Barsoum and Gaddum

The trichloroacetic acid extraction method was employed to determine the blood histamine of 25 experimental and 23 control rats and 21 experimental and 21 control guinea pigs.

The results obtained with experimental and control rats are shown graphically in Fig. 1. Histamine values for the blood of control rats ranged from amounts too small to be detected to 3.5 gamma/ml, with a mean of 0.5 gamma/ml. In the infected animals, the values ranged from 0.04 to 5.0 gamma/ml of blood, with a mean of 1.4 gamma/ml. Seventeen of the infected rats showed an increase in blood histamine over the mean value obtained for control animals, while eight gave histamine values equal to or less than the mean for controls.

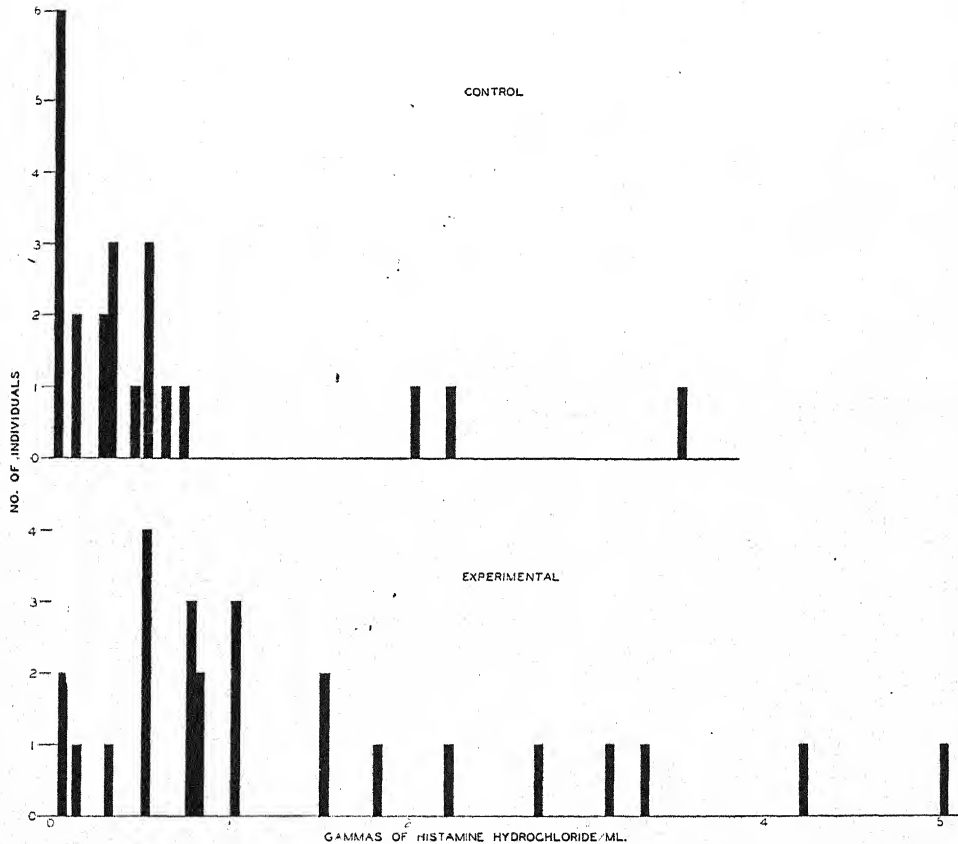


FIG. 1. Blood histamine of rats. Experimental animals infected with approximately 6000 larvae of *Trichinella spiralis* and assayed 16-18 days after infection.

The results obtained with experimental and control guinea pigs are shown in Fig. 2. The histamine content of the blood of the control guinea pigs varied from undetectable amounts to 2.0 gamma/ml with the exception of one animal which gave an unusually high value of 5.0 gamma/ml. The mean for control guinea pigs was 0.7 gamma/ml. Blood histamine values for infected guinea pigs ranged from undetectable amounts to 4.0 gamma/ml with an average value of 1.5 gamma/ml. Seventeen of the infected guinea pigs gave blood histamine values in excess of the mean obtained for the control animals while four gave histamine values equal to or less than the mean for controls.

Determination of Histamine in Tissues other than Blood

In six experimental rats, the histamine content of lung tissue varied from 1 to 12 gamma/gm with a mean of 4.0 gamma/gm and in seven control rats the range was from 3 to 10 gamma/gm with a mean of 5.3 gamma/gm. In 13 experimental guinea pigs, the lung histamine value ranged from amounts too small to be detected to 14 gamma/gm with a mean of 4 gamma/gm while 13 control guinea pigs gave values

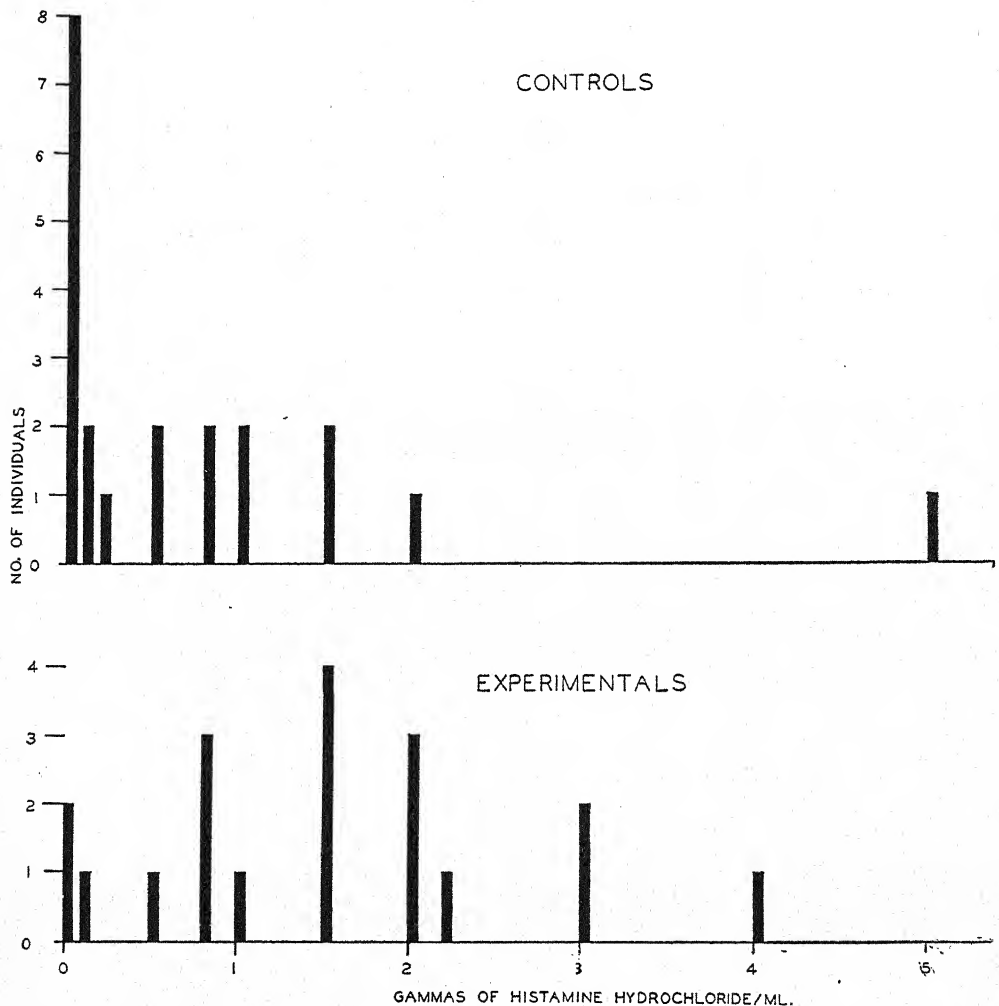


FIG. 2. Blood histamine of guinea pigs. Experimental animals infected with approximately 3000 larvae of *Trichinella spiralis* and assayed 23-26 days after infection.

ranging from undetectable amounts to 6 gamma/gm with a mean of 2.3 gamma/gm. While variation in respect to lung histamine is greater in experimental rats and guinea pigs than in control animals, mean values indicate no significant difference. Histamine content of lung tissue did not seem to be correlated with that of the blood from the same animal.

No significant differences were noted in the histamine content of liver, kidney,

and skeletal muscle of experimental and control rats and guinea pigs. Differences were noted, however, in intestinal tissue of guinea pigs when experimental animals showed marked inflammation of the duodenum as sometimes occurs in acute trichinosis. The intestine of control guinea pigs gave values of 1.0 gamma/gm or less while that of infected guinea pigs with duodenal inflammation gave values up to 8.0 gamma/gm. This difference appears to be significant but the number of animals showing duodenal inflammation was too small to warrant definite conclusions.

Observations on Differential Blood Counts

The results of differential blood counts on experimental and control rats agree fairly well with those reported by Beahm and Downs (1939). Lymphocyte counts on 34 infected rats varied from 48.4 to 84.5 per cent, with a mean of 68.8 per cent, while those of 20 controls varied from 58.5 to 89.5 per cent with a mean of 78.8 per cent. In experimental rats, neutrophile counts varied from 13.4 to 50.3 per cent, with a mean of 24.6 per cent; controls varied from 6.9 to 27.6 per cent, with a mean of 17.1 per cent. There was no appreciable difference between basophile counts in experimental and control rats, the values in both groups ranging from 0.0 to 0.6 per cent. A slight eosinophilia was observed in some of the infected rats, the count ranging from 0.0 to 23.4 per cent, with a mean of 6.4 per cent. Control rats showed eosinophile counts of from 0.6 to 10.8 per cent, with a mean of 4.1 per cent.

Similar results were obtained with guinea pigs. However, eosinophilia in infected animals was more consistent than in rats. Eosinophile counts on 30 experimental guinea pigs varied from 0.3 to 44.8 per cent, with a mean of 10.9 per cent, while 18 control animals gave counts of from 0.0 to 22.6 per cent, with a mean of 4.0 per cent. In both rats and guinea pigs, there did not seem to be a consistent correlation between the eosinophile counts and blood histamine values; some animals with severe symptoms and high blood histamine values gave very low eosinophile counts.

DISCUSSION

Mean values, based upon the above data, indicate that there is an increase of histamine in the blood and possibly certain other tissues of rats and guinea pigs infected with *Trichinella spiralis*. Although the formation of this substance might be expected as a result of the irritation and destruction of tissue by migrating larvae, it may be that the presence of the worms and their metabolites would produce an anaphylactoid condition with concomitant increases in histamine. Code (1939) has found that there is a marked increase in the blood histamine of dogs and guinea pigs during anaphylactic shock.

The results of the present study are difficult to evaluate because of the variability of both experimental and control animals. Furthermore, there is little information concerning the pharmacological significance of slight changes in the histamine content of the blood and tissues and the rapidity with which it may be excreted or destroyed and thus influence the concentration that would be detected at the time of assay.

A comparison of histamine poisoning and trichinosis reveals several interesting parallels. In both conditions, various investigators have reported cardiac insufficiency and lowered blood pressure, edema, inflammation and ulceration of the intes-

tine (in trichinosis, in regions in which adult worms are absent), acidosis and eosinophilia. However, some of these observations have been reported only once or a few times and, in certain cases, have not been confirmed by other investigators.

SUMMARY

The histamine content of the blood and certain other tissues of rats and guinea pigs infected with *Trichinella spiralis* has been investigated. Infected animals showed greater variability than controls but gave higher mean values for blood histamine. Observations on lung, kidney, skeletal muscle and intestinal tissue gave less conclusive results except in the intestinal tissue of infected animals in which inflammation of the duodenum was pronounced.

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A FURTHER DESCRIPTION OF *STEMPELLIA MONIEZI* JONES,
1942, A MICROSPORIDIAN PARASITE (NOSEMATIDAE)
OF CESTODES

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Kudo's monograph on the Microsporidia (Kudo, 1924), lists "Gen. incertum *helminthophthorum* (Keferstein)" under "ambiguous forms" with the following synonymy: *Mucor helminthophthorum* Keferstein, 1862; *Nosema helminthophthorum* Moniez, 1887; *Plistophora helminthophthora* Labbé, 1899. As this list summarizes a rather interesting historical sequence, it may serve as a starting point for an analysis of the earlier reports of microsporidia from cestodes.

REVIEW OF LITERATURE

Bischoff (1855) without naming, described in some detail what was probably a microsporidian from the nematode, *Ascaris mystax*. The dimensions of the spore (which, he said, was motile, and possessed a "keinerlei fadenförmigen Anhang") he gave as circa 8 by 4 micra. Munk (1857) added little but considered the form, which he also found in *Ascaris mystax*, to be identical with that of Bischoff, although non-motile, thus questioning Bischoff's interpretation. Munk did, however, foreshadow later conclusions by Moniez and others, in comparing the effect of the parasite upon the host with the symptoms of the then recently described silkworm disease. Although credit for an original description has been given by subsequent writers to Keferstein (1861), he described, in the present author's opinion, no protozoan parasite at all, but perhaps a fungus, as he and de Bary thought.² His figure, of a hyphate structure bearing "sporangia" at the tips of its many branches, is hardly recognizable as the gross structure of a microsporidian, and indeed resembles strongly a water-mold. Moniez (1887), however, considered that Keferstein, Bischoff, and Munk (l.c.) had all seen the same form. He considered this form to be identical with or very close to his *Nosema helminthophthorum*.³ Moniez, by placing this form in the genus *Nosema* Nägeli, to which the causative agent of the pebrine disease belongs, supported Munk's observation that probably the disease of the nematode, *A. mystax*, was related to the disease of silkworms. Labbé (1899) transferred Moniez's parasite to the genus *Plistophora* Gurley, giving the spore dimensions as 4.2-5.9 by 1.7-2.5 micra. He described "vesicles de 20 μ avec

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² De Bary, to whom Keferstein submitted his material, wrote to Keferstein that "der vorgelegte Pilz zur Gattung *Mucor* Sect. *Hydrophora* gehörte und dass er ihn *Mucor helminthophthorus* benannte. Herr de Bary bemerkt weiter, dass dieser Pilz viele Ähnlichkeit mit einem von H. Hoffmann aus dem Chylusmagen der Biene beschrieben und *Mucor melitophthorus* benannten Pilz habe."

³ He said (p. 1312) "cette espèce vit chez certains Taenias inermes et je l'ai indiquée des 1879, les spores du parasite s'observent en énorme quantité dans les mailles des tissus; elles pénétrant à l'intérieur des ovules dont elles n'empêchent pas toujours l'évolution, et c'est ainsi qu'elles passent à de nouveaux hôtes; ces spores sont ovales et mesurent près de 5 μ sur 2 μ , 5; elles présentent tous les caractères optiques et chimiques du parasite de la pébrine; . . ."

pellicule," and gave a host list to include the "*Taenias inermes*" of Moniez, and *Ascaris mystax* of Bischoff, Munk, and Keferstein.

The attempts by the foregoing authors, especially Moniez, Labbé, and Kudo, to bring together the several descriptions of sporeforming parasites of nematodes and cestodes into one genus do not seem very successful, unless the discrepancies (as shown plainly in Table 1) be dismissed as due to faulty observation. There appears

TABLE 1.—Comparison of data given in cited reports of microsporidia from cestodes and a nematode

Name	No. spores per sporont	Spore size (micra)	Host	Location
Not given (Bischoff, 1855)	?	7.7 by 4.4	<i>Ascaris mystax</i> (nematode)	Seminal vesicles; female genital system
Not given (Munk, 1857)	?	4.2-5.9 by 1.7-2.1	Same	Same
<i>Mucor helminthophthorus</i> ... (Keferstein, 1861)	?	4-5 by 2	Same	Genital system and intestine; hyphate, with sporangia
<i>Nosema helminthorum</i> (Moniez, 1887)	?	5 by 2.5	"Certains taenias inermes"	Tissues, ovaries, ova; in great masses
<i>Plistophora helminthophthora</i> (Kef.) (Labbe, 1899)	?	4.2-5.9 by 1.7-2.5	* <i>Taenia ex- pansa</i> Rud. * <i>T. denticu- lata</i> Rud. * <i>T. bacillaris</i> Goeze	"Parenchyme, organs, genitiaux, ovules"
<i>Stempellia moniezi</i> (Jones, 1942)	1, 2, 4, or 8	4-5 by 1.5-2.0	<i>Hymenolepis anthoceph- alus</i> van Gundy <i>Diorchis reynoldsi</i> Jones, 1943	Parenchyma, mainly in region of excretory canals

* According to Hughes (1941), *Taenia expansa* Rud. = *Moniezia expansa* (Rud.) Blanchard, 1891. *T. bacillaris* Goeze, 1782 = *Hymenolepis bacillaris* (Goeze) Blanchard, 1891. *T. denticulata* Rudolphi, 1810 = *Cittotaenia denticulata* (Rud.) Stiles, 1896.

to be no compelling reason for so dismissing the data of Keferstein and the others; and the author begs leave to suggest that the dismissal of the data of other workers, in order to bring credence to one's own generalization, is at best a practice rather damaging to scientific accuracy. In preference, therefore, to the treatment by Kudo (although, in fairness, his listing of the parasites concerned under "genus incertum helminthophthorum" does not imply his absolute intention of identifying as one form the several listed), the author proposes to let the parasite described by Keferstein remain in the hands of the mycologists as *Mucor helminthophthorus* Keferstein, 1862, to be treated by them as they wish. The other descriptions of the parasites of *Ascaris mystax* and several cestodes (see Table 1), although probably correct as to sub-class and family designation, are inadequate as far as genus and species are concerned. None of them gives the essential information, the number of spores formed from one sporont. Details of spore structure and sporogenesis are lacking. It is therefore impossible to determine whether all or any of the earlier reports actually refer to the present species. In recognition, however, of the fact that Moniez first combined name and description for a microsporidian parasite of cestodes, the present species was named in his honor (Jones, 1942).

GENERIC ALLOCATION

In the genus *Nosema*, in which Moniez placed his "species," one sporoblast gives rise to one spore only. In the genus *Plistophora*, to which Labbé transferred

Moniez's species, the number of spores per sporoblast is sixteen or more. In the genus *Stempellia*, the number of spores per sporoblast is characteristically one, two, four, and eight, all four numbers being found in a single infection (see Kudo, 1924). Since by the description following, the present species is shown to produce one, two, four, or eight spores, per sporoblast, it seems plain that the species should be placed in the genus *Stempellia* Leger et Hesse, 1910.

Stempellia moniezi Jones, 1942

(Figs. 1, 2)

Specific diagnosis: Sporozoa of order Microsporidia Balbiani, 1882, suborder Monocnidea Leger et Hesse, 1910, family Nosematidae Labbé, 1899, and genus *Stempellia* Leger et Hesse, 1910; in parenchymatous syncytium of *Hymenolepis anthocephalus* van Gundy, 1936, and

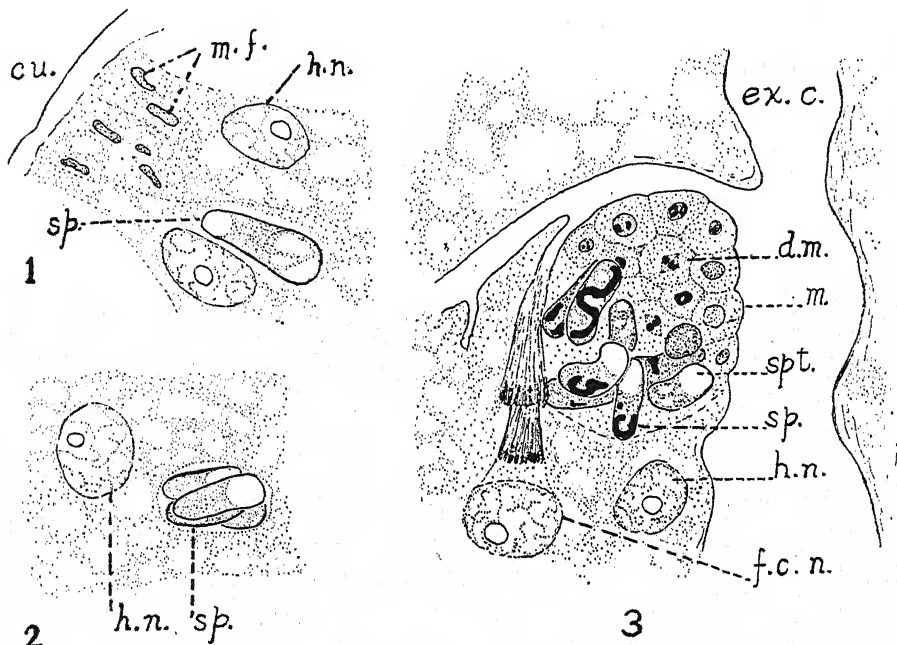


FIG. 1. Spore from monosporous sporont, lying within cytoplasm of a "sub-cuticular cell" of *Diorchis reynoldsi*. (Allen's B-15, iron haematoxylin.)

FIG. 2. Spores from tetrasporous sporont, lying in parenchyma of *D. reynoldsi*. Allen's B-15, iron haematoxylin.)

FIG. 3. Groups of meronts (some in division), sporonts, and young spores, lying against wall of ventral excretory duct of *Hymenolepis anthocephalus*. Flame cell cilia and nucleus shown at left, main canal at right. (Flemming's medium solution, crystal violet stain.)

Abbreviations: *cu.*, cuticle; *d.m.*, dividing meront; *ex.c.*, excretory canal; *f.c.n.*, flame cell nucleus; *h.n.*, host nucleus; *m.*, meront; *m.f.*, host muscle fibers; *sp.*, spore; *spt.*, sporont.

All drawings made with the aid of a Zeiss camera lucida, and enlarged 3,600 times.

Diorchis reynoldsi Jones, 1943, cestodes from the shrew *Blarina brevicauda* Say; spores thin-walled, ovoid to sub-cylindrical, 4-5 μ by 1.5-2.0 μ ; meronts 2.5-3.0 μ , sub-spherical, cytoplasm clear, nucleus single; schizogony produces groups of several to many sporonts; each sporont produces one, two, four or eight spores; spores from octosporous pansporoblast smaller than those from tetrasporous or other sporoblast.

Locality: Charlottesville, Virginia, U. S. A.

Host: *Hymenolepis anthocephalus* van Gundy, 1936; *Diorchis reynoldsi* Jones, 1943.

Type specimens: Slides in author's collection at Miller School of Biology, University of Virginia, University, Va.

MATERIALS AND METHODS

While working in 1941 on a cytological problem concerning certain hymenolepidid cestodes, the author noted, in two cestodes from the small intestine of different specimens of the shrew *Blarina brevicauda* Say, numerous subcylindrical bodies, distinct, in form and staining qualities, from the cestode tissues. Cestodes from about fifteen shrews were examined, but no others infected with protozoa were found.

One of the infected cestodes, *Hymenolepis anthocephalus* van Gundy, 1936, was fixed in Flemming's solution (medium) and stained for karyological contrast with Newton's crystal violet iodine. The other cestode, *Diorchis reynoldsi* Jones, 1943, was fixed in Allen's B-15, stained with iron haematoxylin, and decolorized with picric acid. Sections of all material were cut at 14 micra. Externally, and largely internally, the parasites by both methods showed similar structure. Those stained

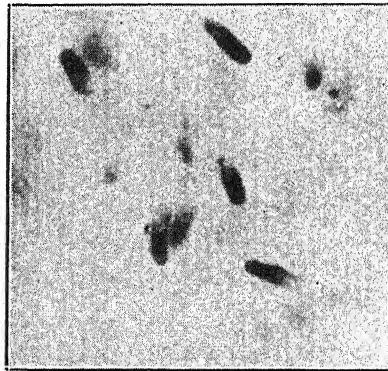


FIG. 4. Photograph of spores lying in the parenchyma of *Hymenolepis anthocephalus*. Magnification circa 1400 \times .

with crystal violet showed more clearly the dense fragments or filaments within the spore (see Figs. 2, 3), while those stained with haematoxylin showed spore vacuoles and cytoplasm more plainly (see Figs. 1, 2). Both preparations showed rather well the developmental stages.

OBSERVATIONS

Locality.—The parasites were found in cestodes from two short-tailed shrews in the vicinity of Charlottesville, Virginia.

Hosts.—The cestode hosts are *Hymenolepis anthocephalus* van Gundy, 1936, and *Diorchis reynoldsi* Jones, 1943. Of about thirty cestodes from shrews examined, including, in addition to the above cestodes several specimens of *Protogynella blarinae* Jones, 1943, only two cestodes were found infected. The manner of infection of the cestode host is not clear, but the possibility of penetration of the cestode cuticula by the sporozoites from spores lying in the mammalian host intestine (perhaps liberated there from the bodies of eaten insect larvae, common hosts of microsporidian parasites) is suggested by Thorne's report (1940) of penetration of a nematode's cuticle by a nosematid parasite, *Dubosquia penetrans* Thorne, 1940. It is likewise possible that ingress into the cestode tissue is by way of the excretory canals, which open in the last proglottid of the cestode. Moniez' (1887) suggestion,

that infection of new cestode hosts by microsporidia occurs by penetration of the ova, is not substantiated by any observations upon the new material.

Habitat.—Meronts, sporonts, and spores are found chiefly in the cytoplasm of the host syncytium surrounding the main excretory canals and their contributing ducts. Often, as in Fig. 3, the parasite is seen lying against the wall of these ducts. In the more heavily infected of the two hosts observed, complete invasion of the whole parenchyma was seen; yet the manner of distribution of the spores in the scolex region corresponded well with the location of the excretory tubules.

Developmental forms.—In general, the parasite conforms to the characters of the genus *StemPELLIA* Leger et Hesse, 1910, as defined by Kudo (1924). The meronts, about 2.5–3.0 micra in diameter, are sub-spherical uninucleate forms occurring in large or small groups resulting from schizogony. At no stage, either meront or spore, are cyst walls of any sort developed, although a differentiating line, like the "wall" of a vacuole, may sometimes be discerned between the host cytoplasm and a group of parasites. No definite shape is assumed by a group of meronts; they generally, as in Fig. 3, conform to the limiting membranes of the host.

The sporonts are a little larger than the meronts, from which they are distinguishable by a somewhat larger and less dense nucleus, and somewhat denser cytoplasm. Each gives rise to one, two, four, or eight sporoblasts. In a large group of parasites meronts, sporonts, sporoblasts, and spores can be seen; in a small group, say, of four or eight cells, all cells are of the same type, either meronts, sporonts, sporoblasts, or spores. It is probable that the large groups are "mixed"; i.e., not descended from one meront; while the small groups should be interpreted as the results of one cell's schizogony.

Spores.—The spores, one, two, four, or eight per sporont, show a slight but observable gradation in size. One individual of a double spore is smaller than the single spore, and one from a group of eight is the smallest. Average spore dimensions are: length circa 4–5 micra by width circa 1.5–2.0 micra. The spores are ovoid to subcylindrical. The wall is quite thin, but appears both resistant and fairly rigid. A vacuole at one end (usually the smaller, see Fig. 1) with a vacuole-like space containing cross-strands (see Fig. 4), or a curled, twisted, or fragmentary body lying in the other, suggests plainly that the spore is similar to the typical microsporidian spore of the family NOSEMATIDAE Labbé, 1899. That is, the spore probably contains a central or subterminal "polar capsule" (the fragments of which are seen in the spores of Fig. 3) with a peripheral girdle of cytoplasm, the sporozoite. Unfortunately, the scarcity of material, and the fact of discovering the protozoa after exhausting the available fresh material, made special techniques for demonstrating a polar filament impracticable. It is possible, indeed, that complete spore development is inhibited in the cestode host, so that only partial differentiation of the filament apparatus occurs. The infrequent occurrence in the literature of reports of microsporidia from cestodes indicates an accidental host-parasite relationship, which may, therefore, be imperfect.

SUMMARY

The reports of microsporidian parasites from cestodes are cited and discussed. The form *Mucor helminthophthorus* Keferstein, 1861, is considered probably not a protozoon, although subsequent writers have given Keferstein credit for the original description and name of what these writers consider a single species, uncertain as

to genus. By analysis of the several descriptions of these parasites, and by a tabular comparison, the discrepancies are brought out, and it appears that several different forms may have been dealt with. The present species can be assigned to none of these earlier forms, hence is described as new. The specific name "*moniezi*" is used in honor of the first observer who both named and described a microsporidian of cestodes. The present species, because of spore-number variation between one and eight, is placed in the genus *Stempellia* Leger et Hesse, 1910.

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COLORADO TREMATODE STUDIES. I. A FURTHER CONTRIBUTION TO THE LIFE HISTORY OF *CREPIDOSTOMUM FARIONIS* (MÜLLER)*

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During the summer of 1939 the life history stages of *Crepidostomum farionis* (Müller) were collected from beaver ponds along East River in the vicinity of the Rocky Mountain Biological Laboratory as follows: cercariae in the gills of the clam *Pisidium* sp.; metacercariae encysted in the abdomen of Mayfly naiads; adults in the intestine of the rainbow trout, *Salmo irideus* (Gibbons). Uninfected Mayfly naiads collected from East River were experimentally infected with the cercariae. Fingerling trout, secured from a nearby fish hatchery, when fed both the naturally and experimentally infected Mayfly naiads yielded immature worms upon examination two to four weeks later. Some of these experiments were repeated in the summer of 1940 with similar results. This represents the first experimental work completed on the life history of *C. farionis*, hence forms an additional contribution to our knowledge of the biology of this trematode.

LIFE HISTORY STAGES

Adults

Either sexually immature or adult specimens of *C. farionis* were found in every one of a total of fourteen trout examined between July 2 and August 8 during the summers of 1939 and 1940. The incidence of infection ranged from 2 to 45. The host fish varied in length from 5 to 8 inches and were caught from two beaver ponds at altitudes of 9850 feet and 9950 feet respectively, adjacent to East River, approximately three miles above the townsite of Gothic, Colorado, where the Rocky Mountain Biological Laboratory is located.

The adult trematodes (Fig. 1) conform with the previous descriptions of Nicoll (1909) and Brown (1927), and meet the specific diagnosis for the species as given by Hopkins (1934), with the minor exception of the ratio between the oral and ventral suckers, in which the former is nearer three-fourths of the latter instead of two-thirds. However, there appears to be great variation here, as in some specimens the ventral sucker is nearly twice the size of the oral. In addition, the posterior testis is slightly larger than the anterior. Six sexually mature specimens killed under a cover glass with formalin, alcohol and acetic acid mixture and stained with Ehrlich's haematoxylin ranged from 1.74 to 2.36 mm in length by 0.556 to 0.678 mm in width. This is a somewhat smaller range than that given by Brown (1927) for his specimens.

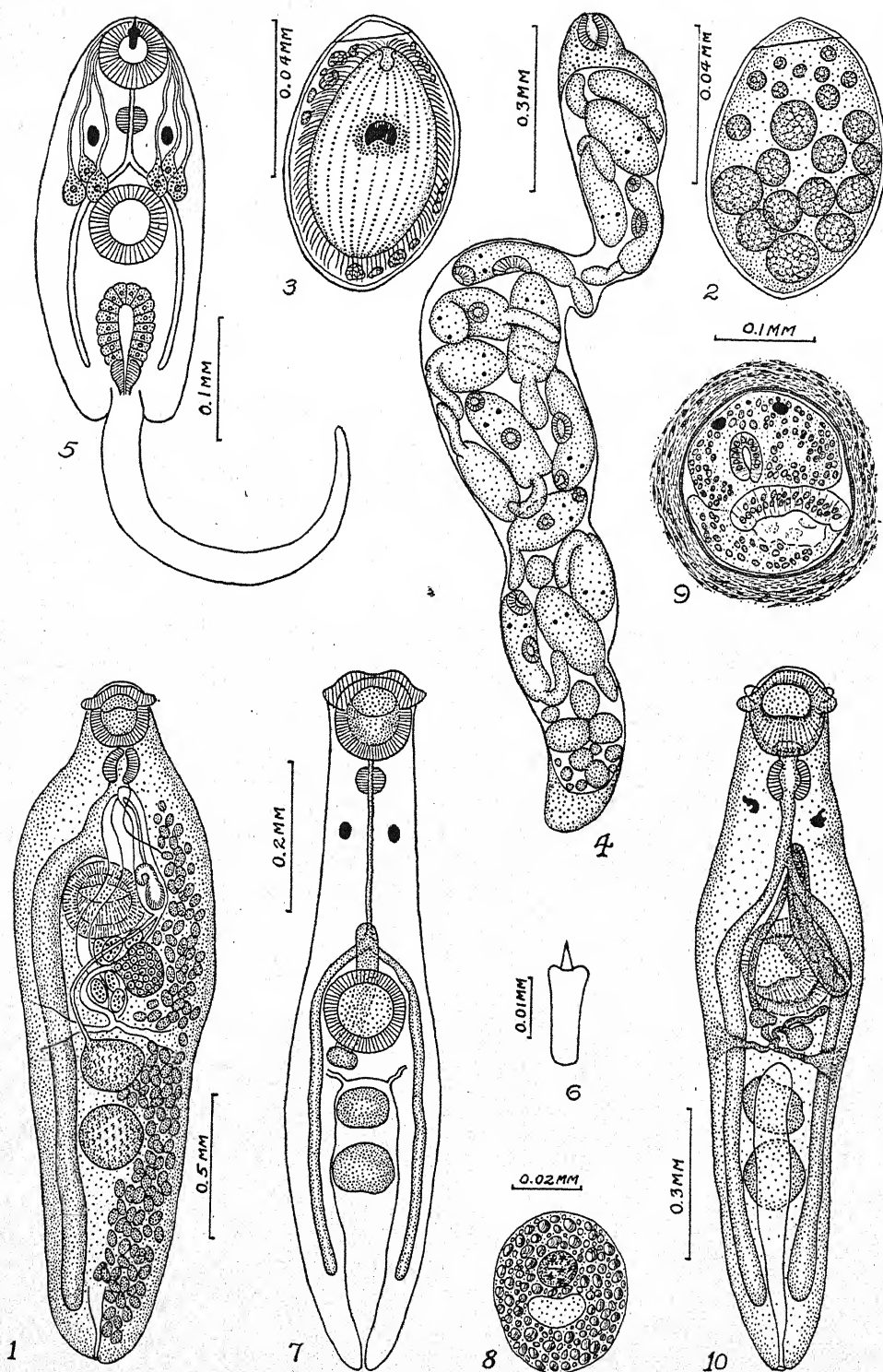
Ova and Miracida

The operculated ova are elliptical in shape (Fig. 2). Ten living ova which had been standing in water for 24 hours varied in size from 65 to 79 μ in length by 50 to 56 μ in width.

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Repeated observations extended over a period of four weeks failed to disclose any free swimming miracidia. During this time active miracidia were observed within certain ova (Fig. 3), many of which subsequently escaped as evidenced from empty ova. The eyespot appears first as two refractive bodies 8μ in diameter which later fuse to form a single mass of pigment (Fig. 3). An eyespot measured in a very active miracidium was 26μ in diameter.

Rediae

The elongated rediae are embedded in the gills of *Pisidium* sp. One redia, filled with cercariae in all stages of development, measured 1.75 mm in length and 0.20 mm at its greatest width (Fig. 4).

Cercariae

Ophthamoxiphidiocercariae were first described by Brown (1927). Three cercariae killed in hot 10 per cent formalin gave the following average measurements in millimeters: length 0.302, width 0.132, eyespots 0.02 long by 0.019 wide, stylet 0.021 long, oral sucker 0.053, ventral sucker 0.053, prepharynx 0.009 in length and pharynx 0.022 in diameter. These measurements are slightly smaller than those given by Brown (1927), who may have been using living material. The living cercaria studied for Fig. 5 was 0.400 mm long by 0.148 mm wide.

The number of infected clams was not large. Of a total of 178 clams collected between July 5 and 15 from the two beaver ponds, but 5 were shedding cercariae.

Metacercariae

Mayfly naiads, *Ephemera* sp., serve as the transfer host of *C. farionis*. The metacercariae encyst in thin-walled cysts, principally in the fat bodies and musculature of the abdomen. They are oval in shape, rather than pear-shaped as reported by Brown (1927) for his material, and the host cysts become impregnated with a brownish yellow pigment as they mature. Ten of the largest cysts obtained from three different hosts varied in size from 0.247 to 0.296 mm in length by 0.234 to 0.270 mm in width. These figures are slightly larger than those reported by Brown

Crepidostomum farionis (Müller)

All figures from permanently prepared slides were drawn with the aid of a microprojector. Cytogenous glands were omitted from Figs. 5, 7 and 10 for clarity.

FIG. 1. Adult *C. farionis*. Killed and mounted in somewhat flattened condition, dorsal view; cystogenous glands omitted on left side, intestine on the right side.

FIG. 2. Egg, living specimen.

FIG. 3. Miracidium of *C. farionis*, living specimen, dorsal view.

FIG. 4. Mature redia of *C. farionis* containing cercariae, living specimen.

FIG. 5. Cercaria of *C. farionis*, living specimen, ventral view.

FIG. 6. Stylet of cercaria, living specimen, ventral view.

FIG. 7. Metacercaria of *C. farionis*, living specimen, fully extended condition, ventral view; excretory granules omitted.

FIG. 8. Epithelial cell of excretory bladder of metacercaria (Fig. 7) filled with excretory granules, living specimen.

FIG. 9. Cyst of *C. farionis* in section from the abdomen of a Mayfly naiad killed and sectioned two weeks after laboratory infection.

FIG. 10. Immature adult of *C. farionis*, prepared slide of specimen recovered from trout intestine three weeks after feeding laboratory infected Mayfly naiads, dorsal view.

(1927). Records kept on a total of 32 naiads collected from the two beaver ponds showed 28 of them to be infected. From 1 to 21 cysts were taken from a single host.

The morphology of the mature papillose metacercaria (Fig. 7) agrees in all of the main features with the description of this stage given by Brown (1927) with the exception of the size. He reported a maximum size of 0.65 mm length by 0.24 mm width for his specimens, while it was not unusual to find metacercariae in this material over 0.80 mm in length. While the suckers are approximately equal in size in this material, as well as in his, they are considerably larger.

Observations on the extrusion of excretory granules through the excretory pore when the metacercariae are subjected to pressure under a cover glass revealed that the excretory granules may be intracellular as well as free in the lumen of the excretory bladder. The extruded cells were usually oval-shaped, had a single nucleus and contained a large fluid-filled vesicle in the cytoplasm. One representative cell (Fig. 8) measured 46 by 40 μ ; another 40 by 30 μ . The contained granules varied in size up to 4 μ . These cells are probably the lining epithelium of the excretory bladder of the cercaria. After encystment the excretory products are deposited in granular form in the cytoplasm of the epithelial cells. The presence of free granules in the lumen of the excretory bladder could result either from their discharge by the epithelial cells, or by the bursting of the latter after becoming heavily laden with granules.

INFECTION EXPERIMENTS TO DEMONSTRATE THE LIFE CYCLE

Infection of Mayfly Naiads

The Mayfly naiads used in these experiments were collected in swiftly running water either from East River or from some of its tributaries. It was considered safe to use the material from this source as but one cyst of *C. farionis* was ever found in the scores of such naiads examined.

The infection of the naiads was accomplished by placing from 3 to 5 of them in a small container with a clam from which cercariae were escaping in the evening and leaving them there overnight. It is assumed that the cercariae gained entrance to the body cavity of the naiads by penetrating the exoskeleton of the abdominal wall. Six naiads thus treated were examined in order to determine the extent of the infection. The results were as follows: number one through six revealed 8, 15, 8, 7, 11 and 9 respectively. These cysts, as in the field infected naiads, were located in the abdomen, either in fatty or muscle tissue.

The metacercariae reach full size from within two to three weeks after the time of infection, as shown by the following data: 6 cysts 16 hours after exposure ranged in size from 0.165 to 0.191 mm in length by 0.148 to 0.165 mm in width; 8 cysts 7 days after exposure ranged in size from 0.213 to 0.226 mm in length by 0.200 to 0.217 mm in width; 8 cysts 14 days after exposure ranged in size from 0.243 to 0.261 mm in length by 0.227 to 0.253 mm in width. The cyst wall, which averaged 8.5 μ in thickness in newly formed cysts, increased to 20 μ within two weeks.

Seven days after the penetration of the cercariae the host cyst has not formed as the cysts readily roll free when the abdomen of the infected naiad is torn apart with dissecting needles. The stylet is still present, but has become dislodged. In one instance it was observed to be repeatedly sucked into the mouth and ejected by

the encysted metacercaria. By fourteen days after infection the host wall is well formed (Fig. 9), and the cysts must be freed with the dissecting needles. The yellowish brown pigment so characteristic of the larger field infected cysts has also begun to form.

Infection of Trout

The experiments to establish the trout as the primary host and the relationship between the metacercarial and adult stages of this trematode were carried on in the summers of 1939 and 1940. The trout used averaged about six inches in length and were secured from the State Fish Hatchery at Pitkin, Colorado, 52 miles from the Rocky Mountain Biological Laboratory. They were hatched from eggs secured from Electra Lake, Colorado. Both field infected and laboratory infected Mayfly naiads were fed to these trout, with similar results.

The results are disappointing in that the writer had to leave the Rocky Mountain Laboratory before the young trematodes had sufficient time to mature in the definitive host. The similarity of structure between both the experimentally reared month-old specimens from field-infected naiads and the three-week-old representatives from laboratory-infected naiads and the sexually mature *C. farionis* is indeed striking and leaves little doubt but that all are representatives of one and the same species. (Compare Figs. 1 and 10.)

Experiments Involving the Feeding of Field-infected Mayfly Naiads

During the summer of 1939 four trout were fed with field-infected Mayfly naiads. Autopsy two to three weeks later revealed a heavy infection in each trout. For example; trout number 4 was fed two naiads on July 23, three naiads on July 28 and three naiads on August 8. The autopsy on August 16 showed 57 immature trematodes of three sizes. The smallest specimens averaged approximately 1.0 mm in length; the largest 1.63 mm.

In an attempt to secure sexually mature adult specimens experimentally, feeding experiments were repeated again in the summer of 1940, but without success. For example, host fish number 8, autopsied 4 weeks after feeding 5 naiads, revealed 17 immature specimens, while host fish number 9, autopsied 31 days after feeding 12 naiads, revealed 97 immature specimens.

Experiments Involving the Feeding of Laboratory-infected Mayfly Naiads

These experiments were performed during the summer of 1939. A total of three trout were used and positive results were obtained in each case. Illustrative of the procedure followed and the results secured is the following example: Trout number 3 was fed on July 27 two naiads infected July 15, on August 4 two naiads infected July 19 and on August 11 two naiads infected July 25. The autopsy on August 12 revealed 31 immature specimens of three sizes, 8 of which appeared to have just recently excysted. No sexually mature adult trematodes were found in any of the three trout. All of the young specimens were found in the intestine.

Although the results are not as conclusive as was hoped, since the experimentally fed larval stages did not attain sexual maturity, they do indicate that the cercaria, metacercaria and adult stages occurring in the beaver ponds all belong to the same

species of trematode. Furthermore, since *C. farionis* is the only adult papillose trematode ever found in trout thus far in these waters, it appears reasonable to conclude it is the species under consideration. The results also show that the metacercariae become infective in the second intermediate host from ten days to two weeks after encystment. This is what one would expect since the growing metacercariae reach mature size in Mayfly naiads in about two weeks after infection.

SUMMARY

1. Additional observations and experiments on the life history of *Crepidostomum farionis* (Müller) have been completed.
2. The life history stages occur as follows: adults in the intestine of the rainbow trout, *Salmo irideus* (Gibbons); cercariae in the gills of the clam *Pisidium* sp.; and metacercariae encysted in the abdomen of Mayfly naiads, *Ephemera* sp.
3. Experiments involving laboratory infection of Mayfly naiads with cercariae and the feeding of both field-infected and laboratory-infected naiads to trout are described. This constitutes the first experimental evidence on the life history for this species of trematode.

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A NEW POLYMASTIGINE FLAGELLATE, *COSTIA PYRIFORMIS*,
PARASITIC ON TROUT¹

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One of the most destructive protozoan parasites of trout and other fresh-water fishes is the flagellate *Costia necatrix* (Henneguy), which is common at hatcheries and fish farms in both America and Europe. This parasite was first described by Henneguy in 1887 and has since received considerable attention from protozoologists. In the spring of 1940 an undescribed species, for which the name *Costia pyriformis* is proposed, was found on rainbow trout, *Salmo irideus*, and brook trout, *Salvelinus fontinalis*, at the U. S. Fish and Wildlife Service hatchery, Leetown, West Virginia.

This organism differs greatly in appearance from *C. necatrix* and there is no difficulty in distinguishing between the two species at a glance. The body is distinctly pyriform, rounded anteriorly and tapering to a point posteriorly (Fig. 1), with the greatest diameter about one-third of the distance from the anterior end. On the ventral side there is a spiral groove which starts on the dorsal side at the anterior end and becomes wider and shallower posteriorly. The flagella arise dorsally at the anterior end of the groove.

Ordinarily, there are two pairs of flagella, one shorter than the body, the other somewhat longer, but, frequently, only one flagellum of each size could be distinguished (Figs. 3 and 4). According to Benish,² this is also true of *C. necatrix*. The flagella are attached to the blepharoplast, which in the living organism appears as a rounded, clear vesicle on the dorsal side, a short distance from the anterior end. The blepharoplast usually stains intensely with iron-hematoxylin, but, occasionally, only a thin outer layer takes the stain, the central portion remaining colorless.

In addition to the blepharoplast, a number of bright, refringent, rounded or rod-shaped bodies can usually be seen in the living organism. These bodies exhibit an active Brownian movement, indicating the fluid nature of the endoplasm. They stain deeply and uniformly with iron-hematoxylin and other chromatin stains, but their nature has not been determined. They differ from chondriosomes in being highly refractive and resistant to reagents. These chromatoid bodies are usually rod-shaped, but vary greatly in length. Frequently, they are arranged in pairs, end to end, and have the appearance of having been formed from a long rod by a median constriction (Figs. 3 and 4). None were observed, however, which could be interpreted as being in the process of division, but it is significant that the paired rods were frequently shorter than the unpaired. The chromatoid bodies usually number from 10 to 15, but occasionally individuals show a considerably higher or lower number. They have no regular arrangement, but are usually confined to the anterior two-thirds of the body, although they are sometimes found near the posterior end. They are absent, however, from the dorsal side of the body, anterior to the blepharoplast (Fig. 2). This appears to be a specially differentiated mobile region which probably aids in the ingestion of food.

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² BENISH, JOHANNES 1937 Untersuchungen ueber *Costia necatrix* Leclercq. Z. Fisch. 34: 755-770.

The nucleus is difficult to distinguish in the living organism, but is easily recognized after staining. It is located near the middle of the body and is of the vesicular type with a rounded deeply staining karyosome in the center. Between the karyosome and the nuclear membrane is a lighter zone containing numerous chromatic granules. A small contractile vacuole (Fig. 3) is located near the nucleus.

Costia pyriformis is considerably smaller than *C. necatrix* with a length of 9–14 microns and a width of 5–8 microns, as compared with a length of 10–20 microns and a width of 5–10 microns for the latter.

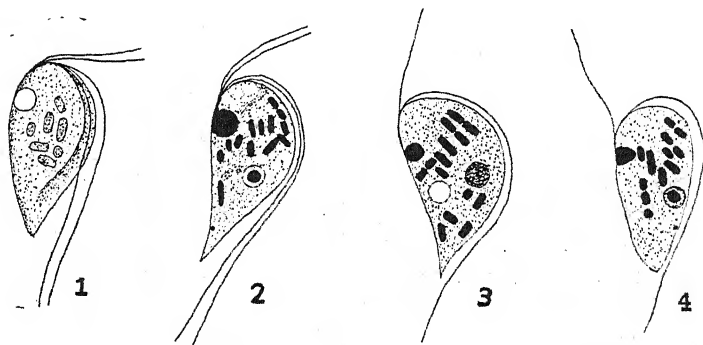


FIG. 1. View of *Costia pyriformis* turned slightly toward the dorsal side to show ventral groove. The blepharoplast appears as a clear rounded vesicle on the dorsal side. Drawn from a specimen killed in osmic vapor, but unstained.

FIGS. 2–4. Side views of *C. pyriformis* stained with iron hematoxylin. The blepharoplast and chromatoid bodies are deeply stained. In Figs. 2 and 4 the nucleus contains a deeply stained karyosome. The contractile vacuole is shown in Fig. 3. All figures $\times 2200$.

The habits of the two species are very similar, although *C. pyriformis* seems to show a greater preference for the gills than does *C. necatrix*. However, it also occurs on the body and fins. Like *C. necatrix*, it is closely attached to the epithelium, but when removed to the slide, becomes dislodged and swims about with a characteristic spiral movement which is very different from that of *necatrix*. So far, *C. pyriformis* has been found on trout only, but it is not improbable that, like *C. necatrix*, it may occur on other fishes as well.

ONE SPECIES OF TREMATODE, *NEORENIFER GRANDISPINUS*
(CABALLERO, 1938) ATTACKED BY ANOTHER, *MESO-*
CERCARIA MARCIANAE (LA RUE, 1917)*

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This paper is an account of the invasion by one species of trematode, *Mesocercaria marcianae* (La Rue, 1917) into the body tissues of another trematode, *Neoreniker grandispinus* (Caballero, 1938) n. comb. The attack occurred within an indigo snake, *Drymarchon corais*, from Florida which died in the Lincoln, Nebraska, zoo in February, 1943. Such direct attack of an adult trematode by a larval stage of another is unique so far as the author can determine. Cort (1915) has reported larvae of *Gordius* (Gordiacea) in the parenchyma of eight specimens of a trematode, *Brachycoelium hospitale*; Fischthal (1942) found a *Paragordius* larva in the parenchyma of one of six specimens of a *Plagioporus* species in a freshwater fish; and larvae of *Cotylurus flabelliformis* develop as hyperparasites in the sporocysts or rediae of other trematodes (Cort, Olivier, and Brackett, 1941).

The snake (*Drymarchon*) was infected with approximately 300 *Neoreniker grandispinus* in the esophagus; with a few *Reniker magnus* Byrd and Denton, 1938; with one linguatulid, *Kiricephalus coarctatus* (Diesing); and with one nematode, *Kalicephalus humilis* Caballero, 1938; as well as with *Mesocercaria marcianae*. Several students assisted in collection of these parasites. At the time of collection, when the probable abundance of mesocercariae was not suspected, only one or two of these were noted. Later, three additional specimens were found in vials containing the *N. grandispinus*. They had perhaps become detached after only partially penetrating the latter. Thirteen specimens of *Neoreniker* had been penetrated by mesocercariae.

A brief history of our knowledge of the trematodes involved is necessary. Caballero in a publication dated March-June, 1938, named a trematode *Reniker grandispinus* from *Drymarchon corais melanurus* in Mexico. In October of the same year, Byrd and Denton (1938) described *Neoreniker drymarchon* from *Drymarchon corais couperi*, originally from Texas. There seems to be no important difference in the descriptions of these two species except, perhaps, in egg size. Byrd and Denton report eggs 34 to 36 by 21 μ in size; Caballero reports 41 to 43 by 20 μ . As a rule, small differences in egg size between species should be supported by other differences because of possible variation among individual specimens. Most of the specimens in my collection have egg sizes of 36 to 39 by 19 to 21 μ , but one specimen, otherwise like the others, has somewhat larger, thinner-shelled eggs measuring 41 to 44 by 20 to 24 μ . Therefore, this species seems to have a rather wide variation in egg size, although the eggs within a single specimen are usually very uniform in size. The lengths of body spines are altogether too variable to be considered of specific value. While there might be some question regarding the validity of the genus *Neoreniker* Byrd and Denton, 1938, as distinct from *Reniker*, the genus is tentatively accepted here. Accordingly, the name of the

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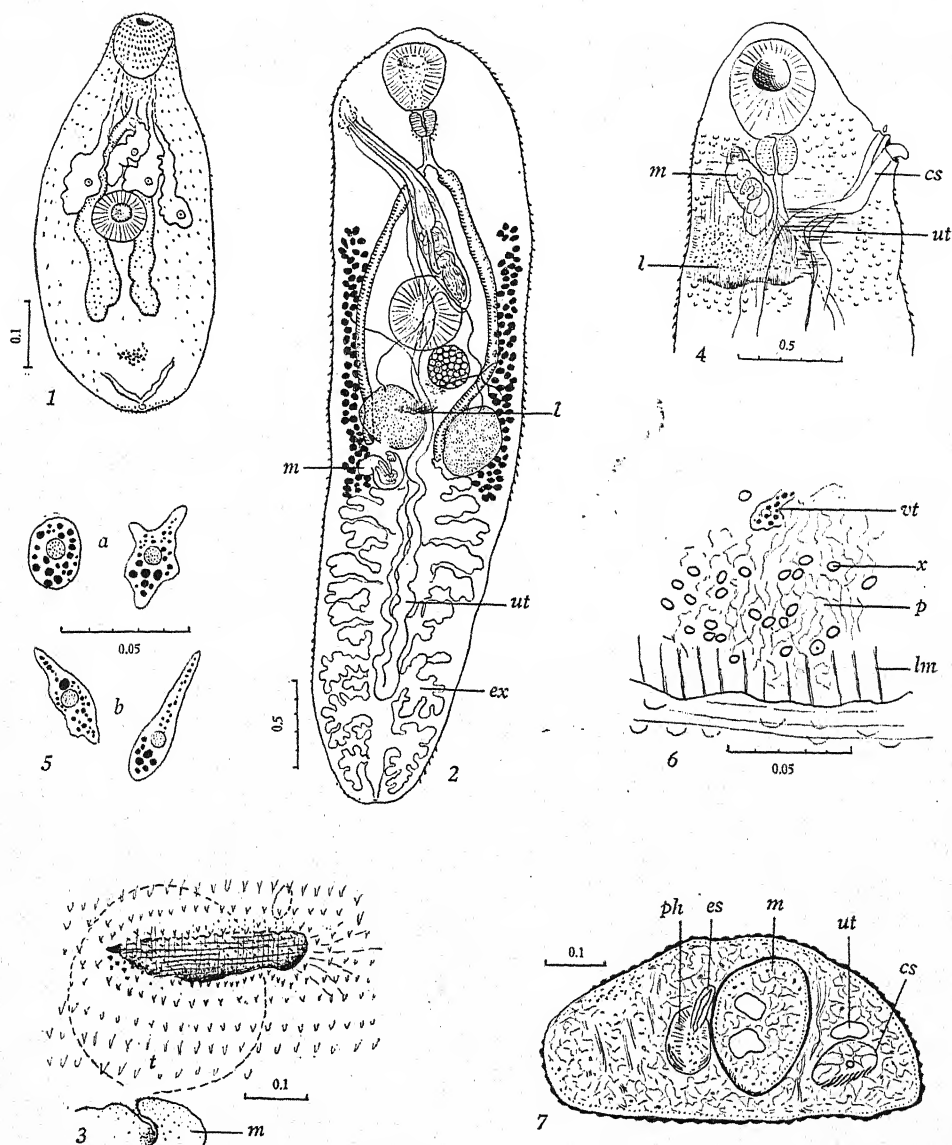


FIG. 1. *Mesocercaria marciana*. Ventral view. Specimen found in vial with collection of *Neorenilifer*.

FIG. 2. *Neorenilifer grandispinus*. Dorsal view, showing mesocercaria embedded in parenchyma posterior to left testis, and place of entrance dorsal to testis. The membrane of the testis had been perforated by the mesocercaria, probably at the time of killing.

FIG. 3. Enlarged view of wound caused by entrance of the mesocercaria shown in Fig. 2. In this case, the muscle layers of the body wall have apparently regenerated.

FIG. 4. *Neorenilifer grandispinus*. Ventral view of anterior portion showing embedded mesocercaria and lesion produced by its entrance.

FIG. 5. Vitelline cells associated with wounded areas of the parenchyma; (a) two cells from wounded areas; (b) two cells from the parenchyma near yolk glands.

FIG. 6. Enlarged portion of posterior part of wounded area shown in Fig. 4, showing vitelline cell, ovoid bodies on surface of parenchyma, broken ends of longitudinal muscles, and posterior edge of the wound.

trematode is considered to be *Neoreniker grandispinus* (Caballero, 1938) with *N. drymarchon* as a synonym. Caballero's figure (his plate III, Fig. 3) is labeled "*Renifer longispinus*" which must be considered as a second synonym of *Neoreniker grandispinus*.

A morphological feature not noted in the descriptions of this trematode is the branching of the Y-shaped excretory vesicle. The unpaired stem gives off lateral branches which may also be branched (Fig. 2). The main stem forks just posterior to the acetabulum and the crura extend only a short distance anterior to the acetabulum.

In 1917, La Rue described *Cercaria marciana*, an immature distome, found in large numbers in the tissues (especially in the tail region) of a garter snake, *Thamnophis marciana*, from Texas. Cort (1917) found the same trematode in the tissues of tadpoles and in the digestive tract and body cavity of garter snakes at Douglas Lake, Michigan. He was able to infect garter snakes experimentally but found that the trematode did not develop beyond the stage found in the tadpoles, a stage then called "agamodistome," now, more specifically, a "mesocercaria." The fork-tailed cercaria of this mesocercaria was later described by Cort and Brooks (1928). It is a holostome cercaria developing in *Planorbis* species at Douglas Lake. Bosma (1934) described the life cycle of a related mesocercaria, *M. mustelae*, as requiring four hosts: a snail, a tadpole or frog, a small mammal, and a carnivorous mammal, in which the metacercaria developed into *Alaria mustelae* Bosma, 1931. In 1938, a similar life cycle was described by Olivier and Odlaug for *Alaria intermedia* Olivier and Odlaug, 1938. Cuckler (1940) fed *Mesocercaria marciana* and *M. intermedia* to rats and mice and found that while some of the trematodes became encysted metacercariae, most of them migrated to the lungs where they became unencysted metacercariae which later reached the trachea, were swallowed and finally reached maturity (*Alaria* spp.) in the intestine. Therefore, only three hosts seem to be necessary although four may be commonly involved. Cuckler's work experimentally completed the life cycle of *Mesocercaria marciana* to the adult "*Alaria* sp." in a mouse. Probably the adult could also develop in some other mammal eating a mouse containing metacercariae. The discovery of *Mesocercaria marciana* in a trematode in an indigo snake indicates that this mesocercaria might live temporarily in almost any kind of host.

The indigo snake had been in the Lincoln zoo about a year. So far as is known it had eaten only twice: a mouse several months previously and, about two weeks before its death, a garter snake. Its infection with *Neoreniker* had almost certainly been acquired in Florida since all 300 specimens were fully mature and approximately the same size. The mesocercariae were presumably recently acquired because they

FIG. 7. Cross-section through a *Neoreniker* infected in pharynx region, showing relative size of mesocercaria, and absence of tissue reaction.

All drawings were made with the aid of a camera lucida.

cs	cirrus sac	p	parenchyma
es	esophagus	ph	pharynx
ex	excretory vesicle	t	testis
l	lesion or wound	ut	uterus
lm	longitudinal muscles	vt	vitelline cell
m	mesocercaria	x	ovoid body of unknown origin

were in the process of migration. Although either the mouse or the garter snake or both could have been the source of the infection, the garter snake seems more probable.

The infection in *Neoreniker* was discovered in one of the last specimens collected while it was being killed under a coverglass. The mesocercaria was squirming about as if in a cyst in the parenchyma. It was completely imprisoned. The killing solution soon penetrated its tissues so that only a brief observation of its living condition was possible. Later, 12 additional specimens were found infected while a number of others had been attacked and apparently partially penetrated in one or more places. There is little evidence as to whether the attacks were quite accidental, or selective. The massive numbers of the *Neoreniker* and the small proportion affected suggest the former. But the fact that the mesocercariae should persist in scraping away at a surface armed with such formidable spines as those of *Neoreniker*, and the further fact that one specimen was attacked in five different places, another in three places, suggest either some sort of attraction, or unusual persistence. Thirteen (about 4%) of the 300 specimens contained mesocercariae. Yet 4 (about 30%) of the 13 contained double infections. In 9 cases, the mesocercariae were in the anterior half of the body; in 4 cases in the posterior half. Five infections were close to the pharynx near the central nervous system. In all 4 cases of double infections the two mesocercariae were not far apart.

EFFECTS ON THE MESOCERCARIAE

A total of 17 mesocercariae were observed within the parenchyma of 13 *Neoreniker*. All specimens were completely embedded and unencysted in the parenchyma, sometimes stretched in one direction, often bent at least once. Their size and stage of development corresponded to those of 5 specimens secured outside the trematodes. In general, therefore, no evident effect was seen on the mesocercariae and no advantage to them by the hyperparasitism could be deduced. However, one specimen (in one of the doubly infected *Neoreniker*) was dead. It was almost completely transparent, its outlines and suckers being barely visible; its tissues had disappeared as if by solution; an inflated vesicle-like structure was interpreted as one of the intestinal ceca. There was no evidence as to the cause of the disintegration.

EFFECTS ON *NEORENIKER*

Effects on the host trematode were more evident. Most of them, however, were limited to the surface area of penetration. In almost all cases, the place of penetration could be detected not very far from the body of the mesocercaria. In one of the double infections, one larva had entered the ventral surface just anterior to the acetabulum and ploughed backward dorsal to the left border of the acetabulum until its anterior end was on a level with the posterior edge of the acetabulum (a distance of 0.645 mm). Here it met (being almost in contact with) the anterior end of another mesocercaria which had penetrated the dorsal surface opposite the oötype just posterior to the opening of Laurer's canal and traveled to the left about 0.36 mm. Strange rendezvous!

Once embedded in the parenchyma the mesocercariae seemed to stimulate little or no reaction except in two cases where severe damage was done to reproductive organs. Even in the case of one specimen implanted directly in or on the central nervous system near the posterior border of the oral sucker, no injury was evident.

Two specimens were effectively castrated by the mesocercariae, in one case both egg and sperm production being suppressed. Probably the most easily damaged organs of a trematode are the vitelline glands. In one specimen, a mesocercaria had penetrated longitudinally through the middle of one of the vitelline fields. Excessive vitelline secretion had flooded the entire length of the uterus so filling it that only a few highly abnormal, abortive eggs occurred. The condition was similar to that described by Manter (1927) for *Neoreniker validus* (Nicol). In the same specimen, pressure on the vasa efferentia either by the mesocercaria or by the swollen, yolk-filled uterus seems to have closed these vessels to the flow of sperm cells. At least, the vasa efferentia were well filled with sperm cells posterior to the level of the mesocercaria (near the base of the cirrus sac) but were empty anterior to that level. The seminal vesicle was also completely empty of sperm cells. No eggs or sperm cells could pass from this specimen, although both the ovary and the testes were normal.

The other castration was evidently caused by the mechanical destruction of both testes. The head of the mesocercaria was embedded within the smashed remains of one testis and was surrounded by scattered testicular material. The other testis had been completely destroyed with very few remains of sex cells discernible, but the anterior end of the testicular membrane with the vas efferens attached could be seen. This specimen still had the seminal vesicle filled with sperm cells but its power to function as a male could have continued but a short time.

Most common effects of the parasitism were on the body surface of *Neoreniker*. At the place of attack the cuticula including its spines was scraped or digested away, sometimes over a considerable area. The circular, longitudinal, and diagonal muscles were exposed along the edges of the trauma and usually the parenchyma was exposed in the central area. Such an affected area could almost always be seen not far from the embedded mesocercaria and, in addition, similar abrasions were not uncommon on uninfected specimens indicating unsuccessful, perhaps interrupted, attacks. Considering the extent of the wounds (sometimes almost across the body), there was remarkably little reaction by the tissues. The exposed parenchyma was slightly more granular, with some slight concentration of nuclei, perhaps because of shrinkage of vesicular cells near the surface. The stroma of the parenchyma became more closely fibrous at the surface and a thin membrane-like layer was apparently formed by the cell membranes of parenchyma cells. Frequently, a light brownish color was present in the affected area, and scattered through the parenchyma were a number of small, ovoid particles of light brown color and uniform texture (Fig. 6). The origin and nature of these particles could not be determined. It is thought they were either dead nuclei of parenchyma cells (which they resemble in size) or coalesced particles of shell-like material liberated by yolk cells which had migrated to the spot. It seemed evident that individual cells from yolk glands did migrate to the wound since these cells in amoeboid shape could be found in the parenchyma near the yolk glands and in the affected area (Fig. 5). In the younger wounds they were seen in moderate number but were sometimes apparently lacking especially in the older wounds.

There was no evidence of bacterial infection.

In what seemed to be older wounds, there were continuous layers of longitudinal and circular muscles although mesocercariae had evidently entered at those spots. Therefore, it seems probable that the muscle layers had regenerated. Regeneration

of cuticula was slower and must have occurred, if at all, from the edges of the wound. The small size of the opening of some wounds indicated some such regeneration.

SUMMARY

A specimen of an indigo snake, *Drymarchon corais*, from Florida which died in the Lincoln, Nebraska zoo, contained among other parasites about 300 specimens of *Neoreniker grandispinus*. Specimens of *Mesocercaria marciana* were found embedded and unencysted in the parenchyma of 13 of the *Neoreniker*. The mesocercariae had invaded the other trematode by direct penetration of its cuticula and body wall. Considerable damage was done to the surface area involved in the penetration, with loss of cuticula and muscles of the body wall, leaving the parenchyma exposed. The exposed stroma of the parenchyma seemed to form a fibrous protection while the deeper layers were unaffected; there was a slight accumulation of nuclei and often a deposition of brownish, somewhat refractile bodies of unknown origin; and there was some migration of yolk cells to affected areas. Regeneration of muscle fibers seemed to occur, as well as a slow addition of cuticula to the edges of the wounds. The only internal damage was to the reproductive organs of two specimens. In both cases, the male system had been put out of commission; once by the mechanical constriction of the vas efferentia, in the other case by the destruction of both testes by direct attack of the mesocercaria. One mesocercaria was embedded near the vitellaria and a surplus of yolk material had flooded the uterus and suppressed all normal egg production.

Neoreniker grandispinus (Caballero, 1938) is named as a new combination with the following synonyms: *Reniker grandispinus* Caballero, 1938; *Neoreniker drymarchon* Byrd and Denton, 1938; *Reniker longispinus* Caballero, 1938.

Specimens showing the features described in this paper are deposited in the U. S. Nat. Mus. Helm. Coll., No. 36886.

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STUDIES ON THE BIOLOGY OF THE ARGASID TICK,
ORNITHODOROS NICOLLEI MOOSER¹

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Distribution: Mexico: States of Guerrero, Puebla, Colima, and Jalisco (Mooser, 1932; Brumpt, Mazzotti and Brumpt, 1939; Mazzotti, 1942).

Hosts-Habitats: *Neotoma*, man, and dogs; also found on a rattlesnake in the Zoo in St. Louis, Missouri. Native houses in Mexico.

Feeding time: These ticks feed readily on mice and guinea pigs. Larvae may engorge and detach voluntarily in less than 15 minutes or they may remain attached for several days before completing engorgement. Nymphs and adults completed engorgement in from 15 to 23 minutes.

Molting: June 8 to 11, 1942 eighty-five larvae² completed engorgement on a guinea pig. They were placed separately in shell vials, numbered serially, and accurate records made until all surviving ticks reached the adult stage. This requires individual feeding in all nymphal stages. The ticks were stored at room temperature in humidity jars containing a saturated solution of ammonium chloride.

All larvae molted in 9 to 13 days; first nymphs in 8 to 11; second nymphs in 6 to 20; third nymphs in 7 to 14; fourth nymphs in 11 to 39; fifth nymphs in 12 to 48, and 3 sixth nymphs in 21 days. The spread in molting time was greatest in the fourth and fifth nymphal stages and perhaps more nearly approaches conditions in nature as these ticks detach easily when disturbed and the degree of engorgement influences molting time.

Nymphal stages: As in several other species of *Ornithodoros*, the period of the greatest mortality was during the larval molt. In this series 18 ticks died at the time of the larval molt and three at the first nymphal molt. Sixty-four were reared to adults (37 ♂♂, 27 ♀♀). The first adult, a male, appeared 70 days following the larval feeding and the last, a female, at 160 days. Following the fourth nymphal molt there were 31 ♂♂ and 2 ♀♀; at the fifth, 6 additional males and 22 females, and at the sixth, 3 females.

Oviposition: Observations were made on 21 females, 12 of which were reared and 9 as received from Mexico. Data on the preoviposition period—time from the last engorgement to the appearance of the first egg—indicate a definite trend toward a resting period during the summer months. This is in marked contrast to certain northern species which show a similar period of inactivity during the winter months. As a rule, females that engorged in late autumn began egg-laying in a much shorter time than females engorged in the spring. Of 16 females engorged in November, 11 began oviposition in 15 days or less; the shortest interval was 7 days. On the other hand, 3 females engorged, respectively, in April, May, and July began to oviposit in November, November, and December. The periods were accurately observed in 36 instances; these varied from 7 to 233 days.

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¹ From the Rocky Mountain Laboratory (Hamilton, Montana) of the Division of Infectious Diseases, National Institute of Health.

² The stock from which these ticks were reared was received from Dr. Luis Mazzotti, Instituto de Salubridad y Enfermedades Tropicales, Mexico, D. F.

Number of eggs: Forty-two counts were made on 21 females, with from one to 4 counts for each female. The minimum number of eggs was 184 and the maximum 631. Seventy per cent were above 300, 33 per cent above 400, and 20 per cent above 500. There is also a tendency in this species to oviposit a second time without further feeding. The counts for 10 females ovipositing twice, November and March, were 416 + 301, 251 + 184, 591 + 217, 492 + 268, 563 + 184, 609 + 288, 296 + 315, 608 + 309, 238 + 335, and 382 + 233. As a rule, the number of eggs increases from the first to at least the third or fourth oviposition. As representative of such increases, the number of eggs at the first and third ovipositions, respectively, for 7 females was as follows: 251 - 316; 326 - 476; 344 - 609; 286 - 563; 459 - 631; 266 - 540; 314 - 591.

Hatching time: Thirty-three observations were made. The period between the appearance of the first egg and the first larva varied from 17 to 36 days with less than 25 days in 70 per cent of the cases.

Fertility: The ratio of larvae to eggs is relatively high as compared to the results obtained in some other species of *Ornithodoros* and may not decrease perceptibly, without further mating, during at least 4 ovipositions over a period of 8 months. Females that had no opportunity to mate after early April produced eggs at the third oviposition in late December with 98 per cent hatching. Two females engorged April 4, 1942 and not subsequently exposed to males, failed to oviposit until April 1943. There were 444 and 197 eggs, respectively, with 98 per cent fertility in each case.

Relation to certain infectious diseases: Naturally infected ticks have not been reported. Experimentally, the transmission of the rickettsiae of the spotted fevers of the United States of America, Colombia, and Brazil and transmission through the egg to the first generation have been accomplished (Davis, in press). *Rickettsia diaporica* (American Q fever) is not transmitted by tick feeding but is conserved in the tissues of the tick, as demonstrated by injection, for 223 days and probably throughout the life of the tick. *Pasteurella tularensis* is not transmitted by tick bite but is also conserved in the tissues for at least 207 days, as shown by injection. This organism is definitely harmful to the ticks; molting is delayed and the infected ticks become dwarfed.

SUMMARY

The argasid tick *Ornithodoros nicolleti* found in the states of Guerrero, Puebla, Colima and Jalisco, Mexico, feeds, naturally, on *Neotoma*, man, and dogs. It also feeds readily on laboratory animals. Feeding is rapid with the exception of some larvae which may require several days to complete engorgement. Males mature first. There are from 4 to 6 nymphal stages. Based on 42 counts, the number of eggs varied from 184 to 631. The number of eggs increases from the first to the third or fourth oviposition. There is a tendency to oviposit a second time without further feeding. Fertility was as high as 98 per cent in females that had not mated for at least one year.

Ticks of this species experimentally transmit the rickettsiae of the spotted fevers of the United States of America, Colombia and Brazil with marked facility. Transmission through the egg was demonstrated in Brazilian and Colombian spotted fever. *Rickettsia diaporica* and *Pasteurella tularensis* are not transmitted by bite but are conserved for long periods in the tissues of the tick as demonstrated by injection.

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FURTHER ATTEMPTS TO TRANSMIT *PASTEURELLA TULARENSIS*
BY THE BEDBUG (*CIMEX LECTULARIUS*)*

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Continued unqualified statements in current literature that the bedbug (*Cimex lectularius*) is a vector of *Pasteurella tularensis* suggested the desirability of a method of feeding that would eliminate the possibility of fecal contamination of the host.

In studies reported by Francis and Lake (1922) and by Francis (1927) the infection of mice by bugs was effected only by interrupted feeding of the bug during the infective feeding or subsequently when the mouse tails became covered with bug feces during the biting experiment which lasted an hour. It was also determined that bugs remain infected throughout their lives. Tests for transmission through the egg were not made but microscopic examinations failed to reveal organisms in the reproductive organs. Bogenko (1935) reported infection by bite 15 hours after the infective feeding, and Kamil and Bilal (1938) also have reported transmission by bite. Bilal (1941) further reported transovarial transmission.

Methods: The bugs were placed under a feeding capsule attached to a guinea pig. The characteristic feeding stance of the bug is in a vertical position about the periphery of the enclosed space. Immediately after engorgement, which rarely required more than 5 minutes, the bugs migrated to the top of the capsule and were removed. Following each feeding they were placed in a clean shell vial containing a fresh strip of blotting paper.

Experiment 1: Oct. 30, 1942, forty bugs in the second nymphal stage engorged on a guinea pig ill with tularaemia. Immediately after the infective feeding, 5 of them were ground in saline and injected subcutaneously into a fresh guinea pig, resulting in death from tularaemia the third day.

The remaining bugs, in diminishing numbers, were given 9 test feedings over a period of 4 months with no evidence of infection in any of the 9 host guinea pigs.

Two bugs that were tested by injection following the fourth test feeding caused death of the guinea pig on the fourth day. One, 1, 2, 1, 7 and 14 bedbugs respectively, died following the fourth to ninth feeding. One hundred twenty-five days following the infective feeding the 8 remaining bugs were ground separately in saline and injected into guinea pigs. There was no evidence of infection over a period of 10 days.

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* Contribution from the Rocky Mountain Laboratory (Hamilton, Montana) of the Division of Infectious Diseases of the National Institute of Health.

It must be assumed that these 8 bugs did not acquire *P. tularensis* at the time of the infective feeding or that they subsequently lost the organism. In either case it appears likely that bugs that do not harbor the organism outlive infected bugs. Infected bugs were present through at least 7 test feedings as shown by the injection of washings of the strips of blotting paper on which the bugs rested.

Transovarial transmission: The possibility of infection through the egg was tested by feeding and by subsequent injection of the progeny of the bugs following 5 successive ovipositions. The number of progeny tested was 26, 130, 143, 85, and 96 (ovipositions 1-5 following fourth to eighth feedings). Using the feeding methods outlined above, these bugs were given three test feedings and were injected directly after the last feeding. None of the 15 host guinea pigs or the 5 injected guinea pigs became infected. It seems reasonable to believe some of these were the progeny of infected bugs or, if not, that infection inhibits oviposition.

Infectivity of feces: The infectivity of the feces was tested by the injection at varying intervals of saline washings of the strip of blotting paper.

Thirteen such injections were made. Injections made 10, 28, 111, 119 and 130 days following the infective feeding and 10, 10, 21, 8 and 19 days, respectively, following the last previous feeding were non-infective. Injections made 36, 37, 39, 47, 48, 49, 72 and 88 days following the infective feeding and at intervals varying from one to 16 days following the last previous feeding proved fatal to the injected guinea pigs.

SUMMARY AND CONCLUSIONS

1. Bedbugs (*Cimex lectularius*) failed to transmit *P. tularensis* when a "clean" method of feeding was used, eliminating fecal contamination of the host.
2. Progeny of these bugs resulting from five successive ovipositions failed to infect guinea pigs at three test feedings and by subsequent injection.
3. Bug excrement is infective. *Pasteurella tularensis* seems to shorten the life of the bug.
4. Although infection may take place by fecal contamination, transmission is not effected by bite other than by mechanical transfer during interrupted feeding. Transovarial transmission reported by Bilal has not been confirmed.

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NOTES ON THE LUNGWORMS (GENUS *PROTOSTRONGYLUS*)
OF VARYING HARES (*LEPUS AMERICANUS*) IN
EASTERN NORTH AMERICA*

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Numerous observations on lungworms in wild hares (*Lepus timidus*, *L. europaeus*) have been made at various places in Europe during the past century and a half, but it has been only within the last ten years that parasitological examinations of Varying hares (*Lepus americanus*) have revealed the presence of related metastrongyloids in this hemisphere. During the past four years one of us (Goble) has had the opportunity of autopsying a number of Varying hares from the eastern United States and Canada. It is the purpose of this paper to record observations on the incidence and identity of the lungworms in these animals.

Boughton (1932) found lungworms in 25 of 30 (83 per cent) Varying hares in southern Manitoba. He described these nematodes and named them *Synthetocaulus leporis*. Green and Shillinger (1935a) reported lungworms in 6 of 75 (8 per cent) hares (*L. americanus phaeonotus*) in Minnesota. This survey was conducted by J. F. Bell during the months of April through July inclusive, and the worms were designated as *Synthetocaulus leporis*. In collections made in Minnesota and Wisconsin during October and November of the same year Green and Shillinger (1935b) noted lung lesions in 25 of 40 (62 per cent) hares. These lesions were described and attributed to the presence of lungworms which were called *Synthelocaulus cuniculi*. MacLulich (1937) stated that lungworms in hares (*L. a. americanus*) in Ontario "were only occasionally found and then in small numbers."

Of the animals which were examined in the course of the present study 87 were *Lepus americanus virginianus* from New York and 44 were *L. a. struthopus* from New Brunswick. They were collected during the months of November through March inclusive. The New York specimens came from three different localities in the Adirondack region.

INCIDENCE

Examination of the lungs of 87 hares from New York state revealed the presence of lungworms in 42 per cent of the males (21 of 50) and in 40 per cent of the females (15 of 37). It seems permissible, therefore, to combine the records for the two sexes in determining the incidence of these parasites.

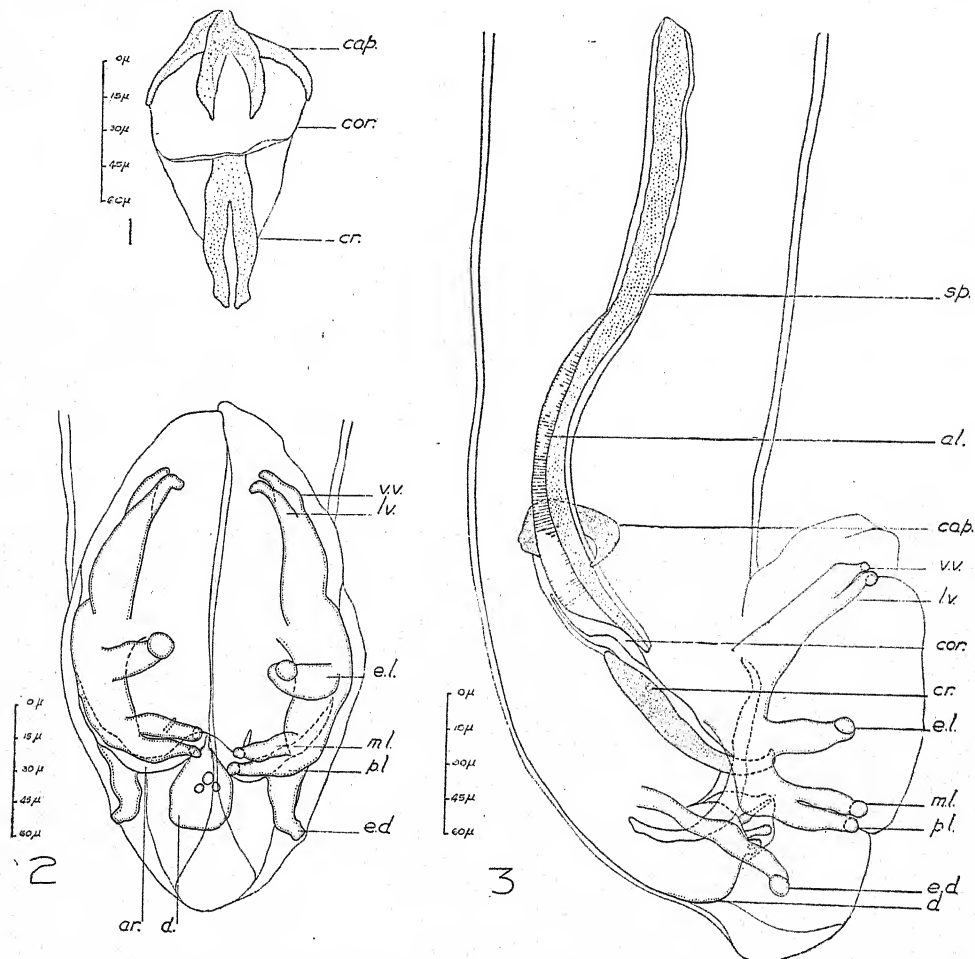
The incidence in the hares from the township of Long Lake in the central Adirondacks was 33 per cent (12 of 36), while that in Dickinson township on the northern edge of the mountains was 48 per cent (16 of 33). In Colton township, which lies between these, 44 per cent (8 of 18) were infected.

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Only 11 per cent (5 of 44) of the hares from New Brunswick were infected.

The number of specimens examined did not warrant analysis on a yearly or monthly basis.



Protostrongylus boughtoni nom. n.

FIG. 1. Gubernaculum of the male: *cap.*, capitulum, *cor.*, corpus, *cr.*, crura.

FIG. 2. Posterior end of male, ventral view with spicules and gubernaculum omitted: *ar.*, sclerotized arc, *d.*, dorsal ray, *e.d.*, externodorsal ray, *e.l.*, externolateral ray, *l.v.*, lateroventral ray, *m.l.*, mediolateral ray, *p.l.*, posterolateral ray, *v.v.*, ventroventral ray.

FIG. 3. Posterior end of male with spicules and gubernaculum: *al.*, spicular ala, *sp.*, spicule.

FIGS. 1-3 made with the aid of the camera lucida.

IDENTITY

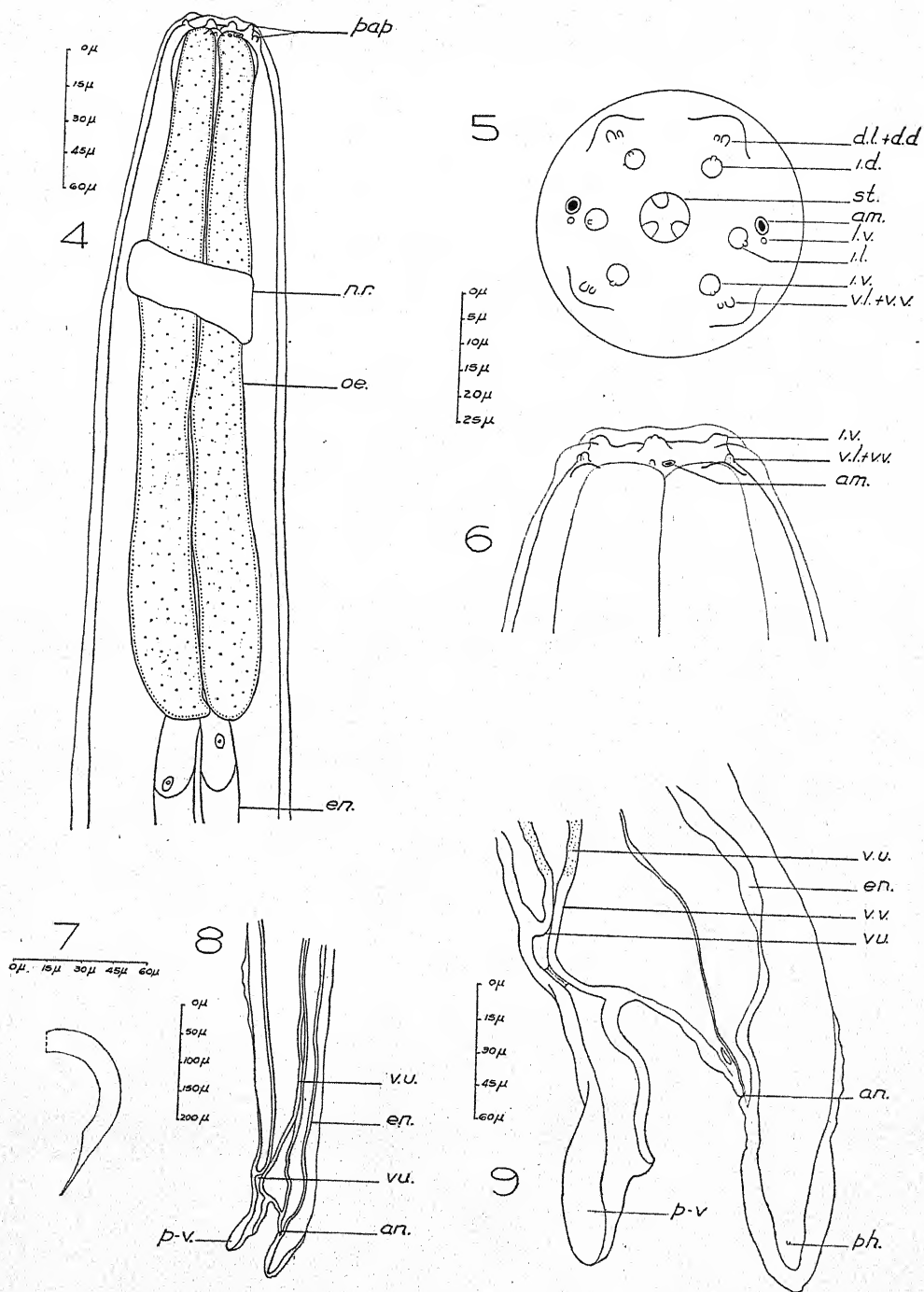
Examination of numerous specimens collected from hares taken in New York, New Brunswick, and Minnesota revealed that these lungworms were morphologically identical with *Synthetocaulus leporis* Boughton, 1932. Dr. Gerard Dikmans of the U. S. Bureau of Animal Industry has examined lungworms from hares from Michigan and Massachusetts and has concluded that they too are probably the same species.

Kamenskii (1905) discussed the identity of the lungworms of European hares and proposed the genus *Protostrongylus* to include the hare lungworms studied by his student Nikulin and related forms described by other authors from ruminants as well as hares. The genus *Protostrongylus* preceded by two years the genus *Synthetocaulus* Railliet and Henry, 1907, which was erected to include the same group of species. *Protostrongylus* has been accepted by most workers since its priority over *Synthetocaulus* was pointed out by Leiper (1926). Russian writers since 1933, however, have reverted to the use of the latter generic name, which had been in general usage from the time it was proposed by Railliet and Henry (1907) to the time *Protostrongylus* was reestablished by Leiper. Kamenskii (1905) did not select a type species for *Protostrongylus*, but Cameron (1927) designated *P. rufescens* (Leuckart, 1865) Kamenskii, 1905, as genotype. Hence this genus appears to be validly constituted, and reversion to *Synthetocaulus* unjustified.

The transfer of the species *S. leporis* Boughton, 1932, from *Synthetocaulus* to *Protostrongylus* would seem, therefore, to be in order if it were not for the fact that Kamenskii proposed the name "*Protostrongylus leporis* sp. inquirenda (an dubia?)" for *Filaria pulmonalis* Frölich, 1802. The specific appellation *cuniculi* inadvertently used by Green and Shillinger (1935b) must therefore come into consideration as a possible name for the species. It appears to us that the acceptance or rejection of this name cannot be definitely determined by application of the Rules of Nomenclature, which are here subject to conflicting interpretation. Article 25 of the Rules states that a specific name shall have a status of availability if a "definition accompanies it by which the species named can be distinguished from other species." The present case resolves upon a subjective opinion as to whether information given by Green and Shillinger constitutes an adequate definition. In all probability forms referred to by them can be identified as Boughton's species. However, other factors contraindicate the use of *cuniculi*, although these are not specifically covered by the Rules. They are: 1) the medium of publication of Green and Shillinger's article was a mimeographed monthly report with a limited distribution and therefore widely unavailable, especially to foreign investigators; 2) the use of *cuniculi* was inadvertent, *leporis* doubtless having been intended; 3) the appellation *cuniculi* was thus not designated as a new name for Boughton's species and was not intended to replace *leporis*. Therefore we reject *cuniculi* as a specific designation for Boughton's species. However, in case difference of opinion should arise among future investigators, we are including for the benefit of the large number to whom Green and Shillinger's account will be unavailable, a direct quotation in the discussion which follows the revised diagnosis of the species under consideration.

In a list of the nematode parasites of the Varying hare in Ontario, MacLulich (1937) gave "*Protostrongylus leporis*. In duodenum . . ."; farther down, "*Synthetocaulus leporis*. Lungworms . . ." appeared. It is apparent that the first of these was either a misidentification or an error in the indication of habitat. In all probability both names refer to the same species of hare lungworm.

In view of the preoccupation of *Protostrongylus leporis* by Kamenskii's species inquirenda, we propose the name *Protostrongylus boughtoni* nom. n., to replace *Synthetocaulus leporis* Boughton, 1932. It seems desirable to refigure the posterior end of both sexes and to make certain other additions to the description of the worm. In the following description our measurements, based on 12 male and 16 female worms, are followed parenthetically by Boughton's.



Protostrongylus boughtoni nom. n.

FIG. 4. Anterior end and esophagus: en., enteron, n.r., nerve ring, oe., esophagus, pap., cephalic papillae.

FIG. 5. Anterior end, en face view: am., amphid, d.l.+d.d., dorsolateral + dorsodorsal papillae, i.d., interodorsal papilla (on lip), i.l., interlateral papilla, i.v., interoventral papilla, l.v., lateroventral papilla, v.l.+v.v., ventrolateral + ventroventral papillae, st., stoma.

Protostrongylus boughtoni nom. n.

(Figs. 1-9)

Synonymy: *Synthetocaulus leporis* Boughton, 1932; ? *Synthelocaulus cuniculi* Green and Shillinger, 1935; ? *Protostrongylus leporis* (Boughton, 1932) MacLulich, 1937 (nec *Protostrongylus leporis* Kamenskii, 1905).

Description: Anterior end of both sexes with six small lips bearing the internal circle of papillae. External circle of ten papillae, dorsodorsals and ventroventrals reduced in size. (Figs. 4-6.)

Male: Length 13-26 mm (23-28), width 160-250 μ (154-176), esophagus 270-360 μ (276-330), spicules 260-320 μ (260-305), capitulum 50-55 μ (about 60, from drawing), corpus 30-45 μ (weakly sclerotized), crura 70-92 μ (68-84). Appearance of bursa, sclerotized arcs, and gubernaculum complex shown in Figs. 2 and 3; gubernaculum illustrated in Fig. 1.

Female: Length 21-36 mm (25-40), width 200-300 μ (154-242), esophagus 325-400 μ (not given), vagina 2-2.4 mm. (1.9-2.4), vulva to tip of tail 160-200 μ (135-194), anus to tip of tail 100-110 μ (about 90, from drawing), provagina 100-154 μ (90-110), eggs 50-70 \times 40-60 μ (65-75 \times 40-50). Body width narrows abruptly from about 125 μ anterior to vulva, to about 65 μ posterior to it. Phasmids present as tiny papillae near tip of tail. Appearance of provagina and adjacent structures shown in Figs. 8 and 9.

Larva: First stage larvae found in lungs and feces are 320-360 μ long and 14-16 μ wide. They have relatively long, straight caudal appendages that differ from S-shaped types reported for several other species of group. Tail pictured in Fig. 7.

Hosts: Varying hare, or snowshoe rabbit, *Lepus americanus* Erxleben.—4 subspecies: *L. a. americanus*, *virginianus*, *struthopus*, *phaeonotus*.

Geographic distribution: Manitoba, Minnesota, Wisconsin, Michigan, Ontario, New York, Massachusetts, New Brunswick.

Protostrongylus boughtoni nom. n. must be distinguished from the other species of the genus with a prominent provagina. It differs from *P. stilesi* Dikmans, 1931, and *P. raillieti* (Shul'ts, Orlov, and Kutas, 1933) Cameron, 1934, in the shape of the distal portions of the crura; from *P. kamenskyi* Shul'ts, 1930, in the length of the spicules and shape of the capitulum; from *P. austriacus* Gebauer, 1931, in length of the spicules and of the vagina; and from *P. coburni* Dikmans, 1935, in the structure of the capitulum and of the externodorsal ray. The latter in *P. coburni* is unique in the possession of a lateral nob or branch at about the middle of its length.

The gubernaculum of *P. boughtoni* is particularly noteworthy in that both corpus and crura appear to have lateral cuticular expansions as illustrated in Fig. 1.

Further reference should be made to the species found in European hares. A careful analysis of the literature on these forms, particularly of the paper by Kamenskii (1905), convinces us that there are at least two species occurring in Europe. Both were considered by Kamenskii, but confused with certain forms in ruminants. A complete analysis of the situation is not within the scope of this paper, but is involved in the complex and confused systematics of the entire genus *Protostrongylus*. Apparently Kamenskii dealt with *P. kamenskyi* Shul'ts, 1930, and with a form called by Kamenskii *P. terminalis* (Passerini, 1884) and by Cameron (1927) *P. commutatus* (Diesing, 1851). There is strong evidence to indicate that the latter species is identical with *Filaria pulmonalis* Frölich, 1802, and we believe that it should therefore be designated *Protostrongylus pulmonalis* (Frölich, 1802) comb. n.

FIG. 6. Anterior end, lateral view showing cephalic papillae.

FIG. 7. Posterior end of larva.

FIG. 8. Posterior end of female (low power): *an.*, anus, *p-v.*, provagina, *vu.*, vulva, *v.u.*, vagina uterina.

FIG. 9. Posterior end of female (high power): *ph.*, phasmid, *v.v.*, vagina vera.

FIGS. 4-9 made with the aid of the camera lucida.

P. boughtoni is distinct from both *P. kamenskyi* and *P. pulmonalis*. An extended analysis of the problems in the systematics of hare and ruminant lungworms of the *Protostrongylus* group is now in preparation.

The following is a direct quotation of Green and Shillinger's (1935b, pp. 91-95) discussion of the lungworms and conditions of lungworm infection in the hares studied by them:

"From all hares showing gross pathology of the respiratory tract, sections were taken for microscopic examination. The gross lesions noted varied to some extent but on the whole conformed to a picture typical of lungworm (*Synthelocaulus* [sic] *cuniculi*) infestation. The most characteristic lesions are easily recognized. There are usually a number of small, hard, discrete, granular areas which can be palpated. They are yellowish gray in color and there seems to be a tendency toward necrosis in the surrounding lung parenchyma. These lesions may be spread indiscriminately over any part of the lung but occur in groups rather than in single foci. When the foci are cut across with a knife, a clear, viscid exudate may be forced from the bronchi by the exertion of slight pressure. The exudate is gelatinous in consistency. If a portion of the lung containing such lesions is macerated in water and floated, it is possible to demonstrate the presence of lungworms in most instances. Pleural adhesions were encountered in two of the hares. In three of the animals showing lungworm foci, the spleen was enlarged to a slight extent. Small abscesses or cysts were noted in the liver in a few cases. Definite caseous necrosis was apparent in the lungs of two hares for which a diagnosis of tularemia was subsequently established either by microscopic examination or by isolation of *Pasteurella tularensis*.

"Microscopic examination of lung from 21 of the 25 hares revealed numerous departures from the normal. In almost every hare there was proliferation of macrophages into the alveoli with varying amounts of pigmentation. A bronchitis and a peri-bronchitis were found in all except two hares. These conditions were characterized by an intense degeneration and subsequent desquamation of the epithelial cells. In many cases the lumen of the bronchi was plugged. The exudate in the bronchi was composed of several elements: erythrocytes, macrophages, epithelial cells, fibrin, and serum. In a few cases the bronchial epithelium showed a preliminary hyperplasia. A waxy substance was also found in some bronchi. A foreign body reaction was associated with the bronchial findings in a few instances. Lymphocytic infiltration was found in only two hares, and in one of these there was an associated necrosis. Cuffing of the vessels was to be seen in four animals. Two types of lungworm infestation occurred. One type of worm was a small organism composed of minute cells containing basophilic nuclei. This type had a definite chitinous sheath. It occurred primarily in the alveoli. The other worm was located in the bronchi and was a typical adult lungworm. The latter worm was a large organism showing the development of various tissues and organs.

"Among the findings in the lungs examined were a number of bodies not previously seen. There were three distinct types of body which showed a tendency toward gradation into each other. The first type appeared a multicellular body and bore a marked resemblance to histoplasma, although staining reactions were not typical. Usually the body consisted of fewer than 5 or 6 cells. In these multinucleate bodies the cell boundaries were indistinct and the cells were joined in such a manner that the periphery was scalloped. The cytoplasm was acidophilic and

filled with acidophilic droplets. The nuclei were situated near the periphery of the cells. They were subject to division as shown by the mitotic figures. Structures of this type varied somewhat as to number of cells, but each cell resembled every other one morphologically. There were also a number of similar single cells about 60–70 [μ] in diameter and definitely round, free in the alveoli of many of the hares. The cytoplasm was acidophilic and contained large acidophilic granules. The nuclei were large, found [?round], and clear but contained very little chromatin, so that they stained faintly. In a number of nuclei were found mitotic figures of such character that they could not be associated with protozoa.

"A second type of body present in the alveoli was similar to toxoplasma and was characterized by acidophilic cytoplasm containing numerous small acidophilic droplets and definitely basophilic nuclei of small size. This body was uniformly multicellular. A third type was a multicellular body containing homogeneous cytoplasm with small, definitely basophilic nuclei.

"Gram-Weigert stains were made of a number of sections showing the various types of unknown body. From study of the sections no evidence was derived to indicate that the bodies were similar in nature to histoplasma or that they were bacterial organisms. They could not be identified as protozoa. Authoritative opinion indicates that they were not worm eggs. The nature of these abnormal bodies is still under investigation."

Microscopic sections of about 100 hares were examined during the course of our study, and bodies satisfying the description given by Green and Shillinger have been observed frequently in the alveoli of infected animals. Comparison of these preparations with similar material from the lungs of deer with protostrongylin infections and sheep with *Muellerius capillaris* infection has convinced us that the bodies in question, contrary to the opinion of Green and Shillinger, represent lungworm eggs in various stages of segmentation. Their "small" worms doubtless were first stage larvae.

Although Boughton correctly noted that the eggs show no trace of segmentation at oviposition, some of his other statements seem to imply that a part of the development might take place in the feces. Our observations indicate that segmentation and hatching normally occur in the alveoli of the lung, as is characteristic of lungworms that deposit unembryonated eggs. The first stage larvae may be found in the alveoli, bronchioles, bronchi, trachea, and alimentary tract. We have never discovered lungworm eggs in the feces, but larvae are readily demonstrable in the pellets when they are present.

SUMMARY

1. The lungworm of the Varying hare in the eastern part of the United States and Canada constitutes a single species originally named *Synthetocaulus leporis* by Boughton (1932). It is here renamed *Protostrongylus boughtoni* nom. n., and a new diagnosis given for it.

2. This species is distinct from the species of *Protostrongylus* occurring in ruminants and also from the two species occurring in European hares, namely *P. kamen-skyi* Shul'ts, 1930, and *P. pulmonalis* (Frölich, 1802) comb. n. (syn. *P. commutatus*).

3. The discussion by Green and Shillinger (1935b) on lungworm infection in hares of Minnesota and Wisconsin is quoted in full. Bodies observed but not iden-

tified by these authors are considered to be lungworm eggs in various stages of segmentation.

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THELASTOMA ICEMI (SCHWENCK), A NEMATODE OF COCKROACHES*

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In September, 1940, the writer obtained some specimens of the American cockroach, *Periplaneta americana* Linn., from Texas through the courtesy of Dr. Donald V. Moore of Rice Institute. Examination of these roaches revealed three species of nematodes. Two of the species were identified as *Hammerschmidtella diesingi* (Hammerschmidt) and *Leidynema appendiculatum* (Leidy). The third species, previously reported only from South America, was identified as *Thelastoma icemi* (Schwenck, 1926) Travassos, 1929.

In January, 1942, after examination of approximately 250 specimens of the roach host from Nebraska, *T. icemi* was recovered in Lincoln; in October, 1942, the worm was recovered from the roach *Periplaneta brunnea* Brunn. in Louisiana. Both of these are new host records. The description of the writer's specimens of *T. icemi* is given below; the male of this nematode is here described for the first time.

Thelastoma icemi (Schwenck, 1926) Travassos, 1929

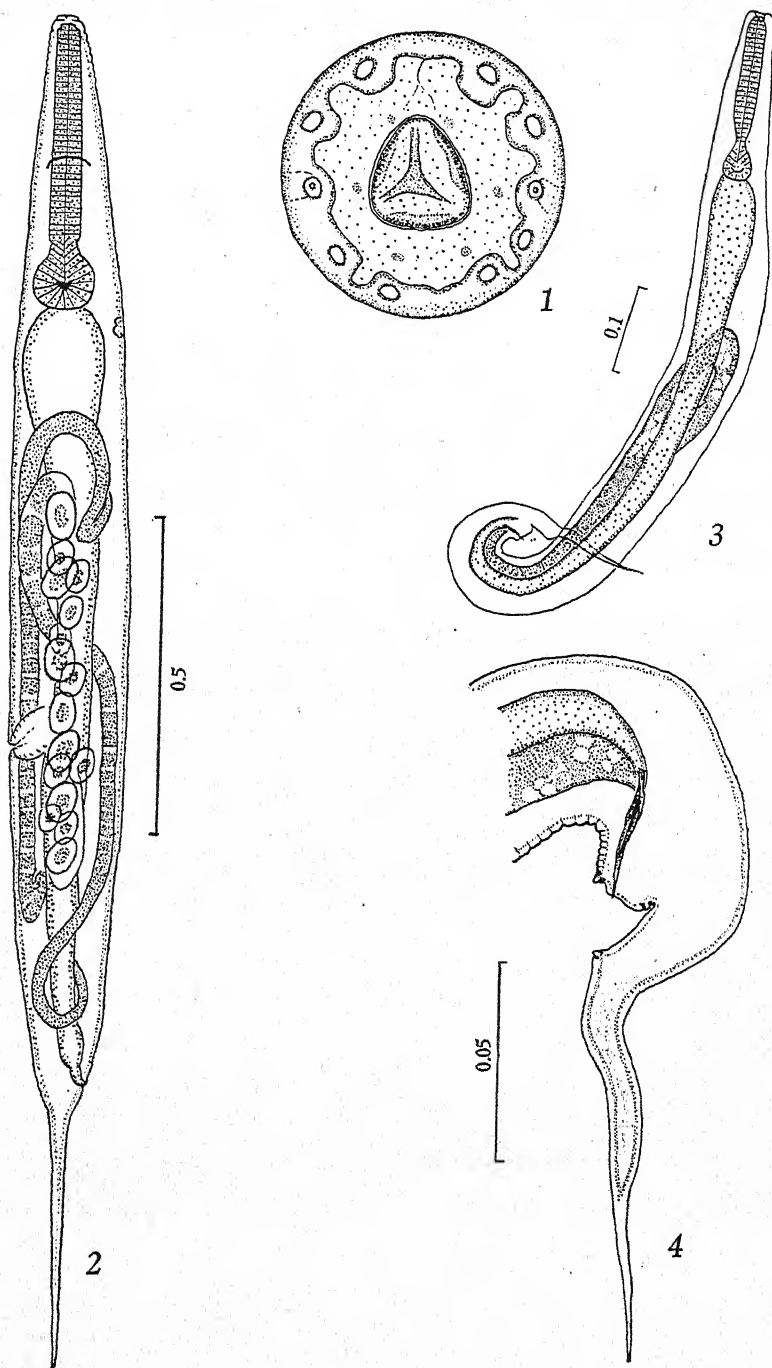
(Figs. 1-4)

With the characters of the genus as given by Chitwood (1932). (Measurements in mm)

Male: 1.1 long by 0.07 wide. Esophagus plus bulb 0.16 long; isthmus not distinct. Anterior portion of esophagus 0.13 long by 0.024 wide (at nerve ring); bulb 0.033 long by 0.034 wide. Nerve ring 0.10 from anterior end. Excretory pore 0.20 from anterior end. Intestine simple; anus 0.18 from posterior end of body. Tail filiform, bearing a pair of ventral preanal papillae, a pair of sublateral postanal papillae and a pair of ventral papillae 0.029 posterior to the anus. One spicule, 0.030 long.

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Thelastoma iceni (Schwenck, 1926) Travassos, 1929

FIG. 1. Head of female, en face view.

FIG. 2. Adult female.

FIG. 3. Adult male.

FIG. 4. Tail of male.

Drawings made with the aid of a camera lucida.

Female: 2.3 to 2.8 long by 0.20 to 0.27 wide. Body distinctly annulated throughout. Tail slender, short, attenuated. Alae inconspicuous. Esophagus consisting of an anterior cylindrical portion separated from the bulb by a short but definite isthmus; esophagus plus bulb 0.37 to 0.42 long. Corpus 0.26 to 0.34 long by 0.035 to 0.037 wide (at nerve ring); isthmus 0.021 to 0.034 long by 0.033 to 0.042 wide; bulb 0.084 to 0.087 long by 0.087 to 0.096 wide, provided with a distinct valve. Intestine pronouncedly dilated at its anterior end. Nerve ring 0.17 to 0.21 from anterior end. Excretory pore 0.38 to 0.47 from anterior end or slightly posterior to base of esophagus. Vulva not salient, 1.22 to 1.30 from anterior end of body; reproductive system amphidelphic. Eggs ellipsoidal, 0.065 to 0.070 long by 0.050 to 0.057 wide, deposited before segmentation. Anus 0.5 to 0.6 from posterior end of body.

The measurements of the female specimens are, with the exception of the diameter of the esophageal bulb, in agreement with the extremes given by Schwenck (1926) in his diagnosis of *T. icemi*.

Chitwood (1932) felt the status of *T. icemi* as a member of the genus *Thelastoma* to be in doubt because the male had not been described, the excretory pore was posterior to the base of the esophagus, and the tail of the female was attenuate rather than filiform as in the type species. The male described in this paper closely resembles the males of *T. bulhoesi* and *T. riveroi* which are listed by Chitwood as valid species. *T. icemi* agrees with *T. magalhaesi* and *T. robustum* in having the excretory pore posterior to the base of the esophagus; such a character appears to the writer to have no more than specific value.

The genus *Thelastoma* was erected by Leidy (1853) when he raised it from subgeneric rank in the genus *Aorurus*. According to my investigations the genus at one time or other contained approximately 22 species whose hosts were reptiles and insects. Walton (1927) created the genus *Thelastomoides* to accommodate the species from reptiles. Recognition of synonymy and transference of species to other genera have reduced the number of species to 18.

Thelastoma icemi most closely resembles *T. magalhaesi* from which it has been adequately distinguished by Schwenck (1926) in his diagnoses of the two worms. *T. icemi* and *T. magalhaesi* differ from other members of the genus which occur in cockroaches in that the excretory pore is located slightly posterior to the base of the esophageal bulb.

SUMMARY

1. The occurrence of the nematode *Thelastoma icemi* in the cockroach *Periplaneta americana* (new host record) from Texas and Nebraska is here reported.
2. A second roach host, *Periplaneta brunnea* (new host record), is recorded from Louisiana.
3. The male of *Thelastoma icemi* is described for the first time.

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THE WANDERING OF *HAEMONCHUS* IN THE SHEEP HOST

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Some degree of wandering in the final host is characteristic of many parasitic nematode species. With *Ancylostoma caninum*, the dog hookworm, which is a skin penetrator, Otto and Kerr (1939), for instance, have used subcutaneously injected larvae as standard experimental procedure to establish intestinal infections. *Haemonchus contortus* does not infect by penetrating the skin, but Ransom and Foster (1920) reported: "The senior writer has observed the larvae of *Haemonchus contortus* (stomach worm of ruminants) in the lung of a guinea pig killed 48 hours after it had been fed a culture of the larvae, which indicates that they are able to migrate from the alimentary tract to the lungs and perhaps do so normally in their life cycle in their usual hosts, sheep, cattle, etc." Ransom seems not to have adduced any further observations on this possibility, although Chandler, Alicata and Chitwood (1941) evidently refer to Ransom and Foster (loc. cit.) when they mention concerning *Haemonchus contortus*: "Although there is no evidence that the worms perform a parenteral migration in sheep, Ransom (1920) showed that they do migrate to the lungs of guinea pigs."

Attempts made by the writer to confirm such an observation by administering larvae by mouth to rats, guinea pigs and rabbits have uniformly failed. Recently, in the course of other experiments on sheep, the demonstration that *Haemonchus* is not a wandering form seems adequately proved. The potential bearing of this fact on explanations regarding the immunization of sheep against this form renders the observations of interest beyond the range of life history knowledge.

MATERIALS AND METHODS

Sheep hosts.—All but five were captured at birth and bottle-fed on cow's milk (Klim) as described by Smith and Ring (1927). When used in the present experiments the five sucklings and four of the bottle-fed lambs had contaminating infections with *Strongyloides papillosus*. The others when used were helminth-free at ages of 7 to 70 weeks, as determined by fecal examinations and cultures (Stoll, 1930).

Infective Haemonchus larvae.—By making collections of feces in a paraffined paper box directly from the rectum of a sheep in which a pure infection of this nematode has been established, larvae could be readily cultured. The "seed" strain during the present experiments was in its 9th–14th passage, and demonstrably pure at every autopsy where *Haemonchus* was used as the solitary infection. Infective larvae were isolated in the Baermann apparatus from cultures 7 to 10 days old, thoroughly washed in several changes of tap water and then exsheathed and rendered bacteria-free as earlier described (Glaser and Stoll, 1940; Stoll, 1940). Such larvae are conveniently referred to as "axenic," Baker and Ferguson (1942) having recently applied this adjective to describe "a living organism that is free from all other demonstrable organisms."

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Injection of axenic larvae.—The larvae during sterilization were in dilute Tyrode solution or well water, the latter at this laboratory approximating a dilute "balanced" salt solution (Stoll, 1940). They were injected within a day after completion of sterilization, and in each case were confirmed as active and vigorous. For intraperitoneal injections, it was found useful for an assistant to hold the animal in an upended position with the head down, causing the viscera to slump toward the diaphragm. The larvae, contained in 4 to 7 ml of suspension, were then injected in the right posterior abdominal quadrant just off the midline. In the case of H38, receiving 1,300,000 larvae, 16 ml was injected. For subcutaneous injections 2 to 4 ml were used. Injections were performed with regard to maintaining sterility, using No. 18 needles. The skin area was sponged freely with alcohol, usually followed by iodine solution, and iodine was used again after injection, particularly to kill any larvae which might have adhered to the skin surface on withdrawal of the needle.

Local skin abscesses resulting from contamination during injection were notably absent, but did occur and persist in one animal, H97, of the intraperitoneal series, which struggled during the injection.

EXPERIMENTS

The procedure employed was to inject axenic infective *Haemonchus* larvae parenterally into the normal host and determine whether the nematodes under such conditions established themselves as parasites in their normal site, the 4th stomach or abomasum.

In preliminary experiments it was demonstrated that axenic exsheathed *Haemonchus* larvae were capable of infecting when administered by the normal route.

1. *Infectivity of exsheathed, axenic larvae given by mouth.*—Infective *Haemonchus* larvae as ingested under natural conditions are characteristically sheathed, that is, the 3rd stage larvae are still enclosed in the cuticular coat of the 2nd stage. Ecdysis normally occurs before the larvae reach the abomasum. Can larvae already exsheathed and rendered axenic before administration by mouth establish an infection?

To ram H9, 13 months old, 5200 axenic larvae were given by mouth. Eggs in the feces were 12 per gram on the 19th, 200 per gram on the 22nd day of infection. Five days later the animal was killed and 322 adult *Haemonchus* (of which 179 were female) recovered from the abomasum.

To wether lamb J59, 7 weeks old, 25,000 axenic larvae were given by mouth. At the first examination on the 13th day the feces gave a positive test for occult blood, which was increased in amount on succeeding examinations until the lamb, severely anemic, died on the 19th day, without eggs of the parasite yet having appeared in the feces. From the abomasum 7020 *Haemonchus* were recovered.

These two animals demonstrate that *Haemonchus* larvae rendered axenic have not thereby lost their ability to infect, and, when sufficient numbers are present in their normal habitat, to produce death.

The cases of 5 animals discussed in the next section (H33, H37, H96, H52, and H97) are also interpreted as evidence of the ability of axenic larvae to normally establish an infection when introduced directly into the rumen. H96 and H97 were especially of interest in this connection.

2. *Intraperitoneal injection of axenic larvae.*—The large size of the rumen in sheep crowds the other abdominal organs and makes the abdominal skin tight. Injection into the peritoneal cavity thus presents the hazard of penetrating the rumen itself. Some of our tests demonstrate this and constitute an experimental error rather than the desired test. However, as placement of the larvae within the abdominal cavity presents them with the most direct opportunity to penetrate the rumen or stomach wall if they can, even one failure to do so after injection into the peritoneal cavity, becomes a fact of considerable significance, from the standpoint of ability of *Haemonchus* to wander or migrate within the host.

H38, a wether 15 months old, was injected with 1,300,000 larvae. Three days later when the animal was killed, numerous "cysts" containing larvae were found in the mesentery and omentum, but the abomasum, though examined with great care, revealed not one *Haemonchus*.

H85, a wether 5 months old, was injected with 286,000 larvae. Six days later when the animal was killed, numerous small pinkish nodules, up to 5 mm in diameter, were found on the omentum and on the rumen wall. The abomasum was examined with great care and not one specimen of *Haemonchus* found.

Fresh preparations of these peritoneal nodules from the two animals showed numerous trapped larvae, but none of them exhibited signs of the first parasitic ecdysis or development into the 4th larval stage. Those from H38, killed 3 days after injection, were for the most part still alive, although some were disintegrating. The proportions were reversed at 6 days in H85. Individual larvae could be seen entirely encased in wandering cells against which they vainly struggled. In these two tests there was no sign whatever that intraperitoneally injected larvae could reach their normal site in the abomasum. Instead they were trapped and being killed off by the peritoneal reaction.

Three sheep were given single intraperitoneal injections and the fate of the infections followed by fecal examination. H81, a ram 4 months old, was injected with 406,000 larvae, and its twin ewe H82 similarly given 81,000 larvae. They remained negative during 11 weeks of fecal examinations. H95, a ram 8 months old, was injected with 454,000 larvae and remained negative during 7 weeks of fecal examinations. All three of these animals became positive when given larvae by the oral route.

The above animals each received a single injection of larvae. Divided doses were also tried. H89, a wether 8 months old, was given a total of 488,000 larvae in 4 doses of about 122,000 each, injected at weekly intervals. Four weeks after the last injection (being 7 weeks after the first), fecal examinations having continued negative, a test dose of larvae was administered by mouth. Through 4 additional weeks no eggs were demonstrable, and then the animal became patent as a result of the oral infection.

The 6 animals above reported represent adequate evidence of failure of intraperitoneally injected larvae to migrate and establish themselves in the abomasum. The records of 5 others are given in which larvae did reach the abomasum, evidently through accidental penetration of the rumen wall by the injecting needle. Two, H33, a ewe, and H37 a wether, each 13 months old, given respectively 209,000 and 355,000 larvae, were positive at fecal examinations on the 34th day, not having been examined earlier. These two animals, the first of the series, were injected while

prone. H96, a ewe 8 months old (and twin to H95 above), was given 108,000 larvae, but it was realized at the time of injection that a hollow organ, presumably the rumen, had been entered. Negative to fecal examination on the 15th day, she was positive on the 17th, and 6 days later reached a count of 35,400 eggs per gram, representing about 22 million per day. H52, a ram 16 months old, was injected with 434,000 larvae. Twelve days later the animal was killed. In an area of about 50 square centimeters in the omentum were numerous firm, pinkish nodules up to 3 mm in diameter. While *Haemonchus* larvae could, with difficulty, be distinguished in these microscopically, none of the worms showed movement or development. On the rumen wall was an area 1 cm in diameter which similarly showed reaction to the presence of *Haemonchus* larvae, as well as evidence of earlier trauma. On examination of the abomasum 60,300 *Haemonchus* were recovered, all in the 4th larval stage, in size up to 4.9 mm long. A 5th animal became positive after "intraperitoneal" injection, under circumstances indicating clearly that such result was direct inoculation into the rumen. H97, a ewe 8 months old, was injected with 112,000 larvae in four approximately equal doses of 28,000 each, spaced weekly. This animal struggled at the time of the first injection, a small abscess later developing at site of puncture of abdominal wall. Negative 15 days after the first injection, H97 showed *Haemonchus* eggs at 17 days. In number these remained very few for a period of 25 days and then rose slightly, reaching 600 eggs per gram 6 weeks after the initial injection (3 weeks after the 4th injection) and 2200 per gram a week later. In the case of H97, evidently there was some injection directly into the rumen at the 1st and 4th injections. However, failure of the egg count to increase until an interval appropriate for the 4th injection, indicated no increase in the abomasal infection until then, i.e., no penetration into the stomach by worms reaching only the peritoneal cavity on the 2nd and 3rd injections. None of these five exceptional cases furnish indubitable support to the successful wandering of intraperitoneally injected larvae to their home in the abomasum, as evidence in each instance suggests instead direct inoculation of larvae into some anterior portion of the gastro-intestinal tract.

3. *Subcutaneous injection of axenic larvae.*—Axenic larvae were next introduced subcutaneously, in order to avoid the occasional accidental inoculation into the anterior portion of the alimentary tract which occurred when injecting them intraperitoneally. In 24 of these tests the wool-free, inner aspect of one or both hind legs was used as the site of injection; in 2 additional lambs the larvae were given subcutaneously in the neck region.

TABLE 1.—Results of single subcutaneous injections of 26 sheep and lambs with axenic *Haemonchus* larvae. One was negative for nematodes at autopsy 7 weeks after injection; each of the other 25 became positive after normal *Haemonchus* infection by mouth

No. of animals	Age	Larvae injected	Examination periods after injection : weeks	No. of fecal examinations per animal	Results
3 sheep	12 months	119,000–147,000 (Av. 133,000)	7–9 (Av. 8)	12–17 (Av. 15)	Negative
5 lambs	20–23 weeks	55,000–80,000 (Av. 65,000)	3–11 (Av. 7)	4–7 (Av. 5)	"
4 "	14–19 "	49,000–61,000 (Av. 53,000)	6–11 (Av. 9)	4–7 (Av. 6)	"
12 "	6–9 "	24,000–47,000 (Av. 39,000)	5–9 (Av. 8)	7–16 (Av. 9)	"
2 "	2–3 "	28,000; 40,000	4; 8	3; 9	"

The results are summarized in Table 1. One of the lambs, J49, 7 weeks of age when injected with 21,000 larvae in 2 ml suspension in both right and left legs, was negative at weekly fecal examinations for 7 weeks. Then killed, no *Haemonchus* were found, the stomach being examined with special care. The remaining 25 animals were subsequently exposed to *Haemonchus* infections by mouth and became positive. Preceding the patency induced by the oral infections, they remained negative to fecal examinations for 3 to 11 weeks. During this non-patent period they were examined 220 times by floatation or culture, 3 to 17 times per animal.

In addition to these 26 tests, a month-old Guernsey bull was given 145,000 axenic larvae subcutaneously, aliquot portions being injected under the skin of each hind leg and the neck. The calf likewise remained negative to fecal examinations.

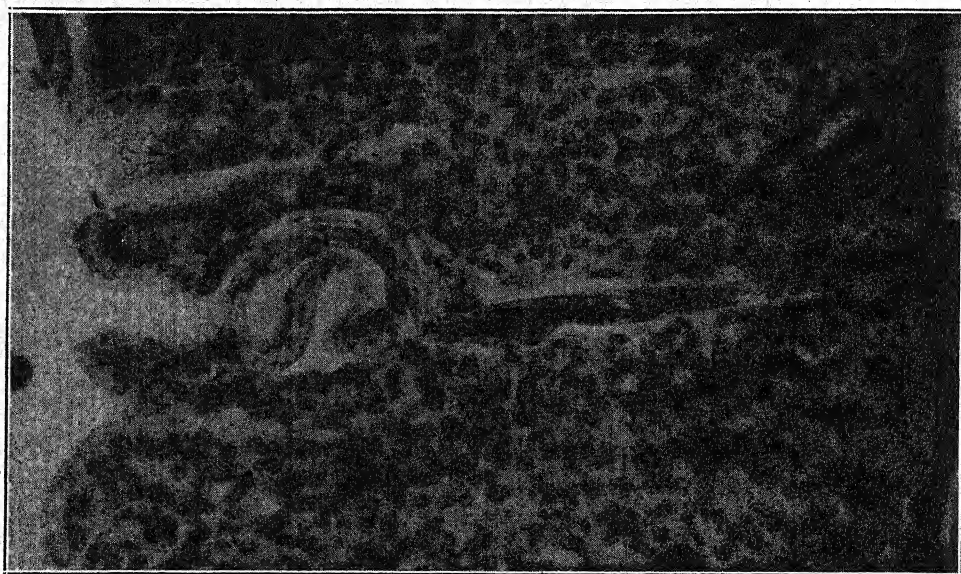
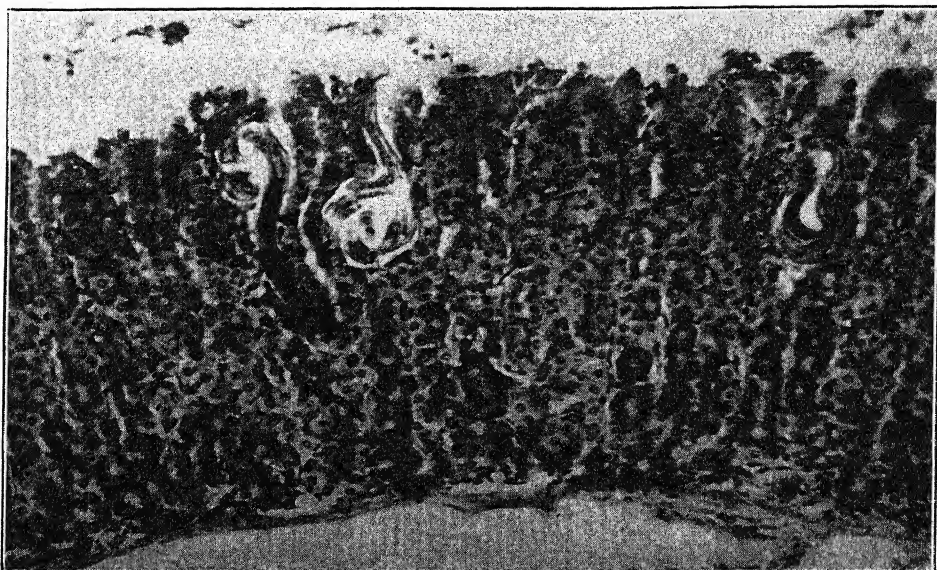
This consistent series of negative results following the injection of axenic larvae by the subcutaneous route into susceptible, normal hosts must be accounted a rigorous test of their inability to wander.

Pseudo-Migration by Haemonchus in the Abomasum

While *Haemonchus* is not to be classed as a wandering nematode in its final host, there is a minor degree of migration during the early establishment of larvae in the abomasum after normal infection by mouth. This pseudo-migration is undertaken by the 3rd stage larvae, which have usually lost their 2nd stage sheaths by the time they reach the 4th stomach. Here, in Veglia's (1916) words, "they lodge between the minute processes of the mucosa, where they shelter without actually piercing the mucosa."

Some favorable material obtained from animals reared helminth-free and killed within 2 days after being given a single heavy infection of larvae by mouth, has permitted a better understanding of this first phase of abomasal parasitism by *Haemonchus*. A portion of an abomasal "leaf" examined in physiological saline promptly after killing of the host, shows striking differences in animals infected 12, 27, and 40 hours previously. In a 12-hour infection the larvae are still predominantly on the surface of the mucosa. They may be seen there with the aid of the low power binocular microscope and they may be washed off rather easily. In a 27-hour infection, some larvae are visible on the surface, but most of them are beneath it. Within a few minutes in warm saline they can be seen escaping from a cut edge in great numbers. In such stomach tissue fixed promptly and sectioned, they are characteristically observed in the gastric pits, which receive the tubules of the glands. The larvae, usually coiled like a watch spring, are somewhat larger than the pits and their presence increases the size of the cavity each occupies. A few show the tail extruded to the musocal surface. In general, however, they are entirely hidden in the upper third or half of the mucosa, and may extend laterally a distance of 40 to 60 μ , more or less parallel to the surface.

While most of the larvae appear to remain coiled at the level of the gastric pits, occasionally one is seen to have penetrated the neck of a gland and gone deeper into its lumen. Of 74 unselected larvae traced from section to section individually, 12 or 16 per cent, had done this. In such case the anterior end of the larva may reach as far as the fundus of the gland, or even go on to nearly touch the muscularis mucosae. The larvae show no predilection for blood vessels at this stage. There is no extravasated blood near them, and little or no tissue reaction, which may be



FIGS. 1-2. Pseudo-migration of *Haemonchus contortus* larvae in abomasum of sheep 27 hours after normal infection by mouth. This is the 1st parasitic stage, passed in the gastric pits, but here the 1st parasitic ecdysis has not yet occurred. The three larvae in Fig. 1 (upper figure, with gastric lumen above; $\times 230$) showed in neighboring sections no penetration deeper into the mucosa than here photographed. This characterizes the position of 84 per cent of those observed. The larva in Fig. 2 (lower figure, with gastric lumen at left; $\times 400$) has the anterior end thrust deep into the tubule of a gastric gland.

Zenker fixation; haematoxylin-eosin stain; sections 9 microns. Photomicrographs by J. A. Carlile.

accounted additional evidence that they tend to accommodate themselves to the naturally available openings. Fig. 1 shows three larvae in section at 27 hours after infection, and Fig. 2 a single larva from the same host, the anterior end of which has penetrated deeply into a glandular tubule. In favorable sections at 27 hours the retraction of the 3rd stage sheath can be seen at the anterior tip of the larva, with the primitive capsule of the 4th stage larva already formed within, as in Fig. 3.

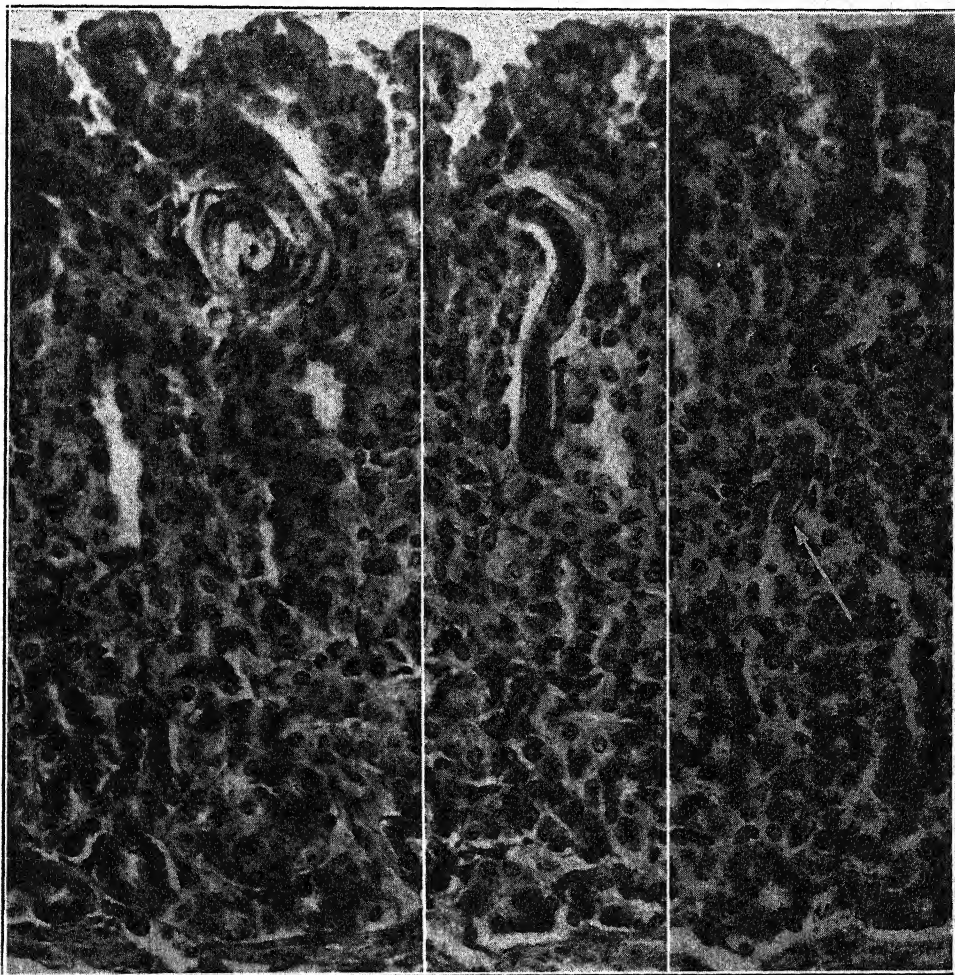


FIG. 3. Three successive sections (9 microns each) from abomasal leaf of sheep 27 hours after oral infection with *Haemonchus*. In this specimen the 1st parasitic (3rd larval) stage is near completion, separation of the sheath having begun at anterior end of larva (arrow).

Magnification $\times 400$; Zenker fixation; haematoxylin-eosin stain. Photomicrographs by J. A. Carlile.

The photomicrographs are from a sheep which received an oral dose of 1,500,000 infective larvae. While the mucosa was extensively parasitized by this huge number of organisms, the number of gastric pits which had been entered by larvae represented only about one-thirtieth of the number of such openings. In a 40-hour infec-

tion occasional embedded larvae can still be observed, but the majority are again at the surface, and these are now characteristically young 4th stage larvae.

It will be recalled that the first parasitic ecdysis and emergence of 4th stage larvae can be obtained in vitro (Stoll, 1940) in the absence of nutrients. On this account it is doubtful whether Veglia's statement that "as soon as the mature larvae have shed their skins [i.e., 2nd stage sheaths] they begin to feed" can be regarded as established. The short migration into the gastric glands is more likely a requirement for the first parasitic ecdysis under conditions of lowered oxygen tension, as demonstrated in vitro. During this pseudo-migration there was, as noted, no evidence of blood vessels being traumatized, and stomach contents of a sheep—even after a 40-hour infection—gave no positive test for occult blood, although the infection was established with 535,000 larvae. The in vitro observation here parallels that of Veglia: "It appears consequently that but little or no growth occurs in the third stage. The object of this period of life seems to be the formation of a mouth apparatus adapted to piercing the mucosa of the stomach and thus to enable the larva to resume its parasitic life as a blood sucker." There is no difficulty in demonstrating blood in the abomasum when the 4th stage larvae or young adults are present in numbers.

DISCUSSION

As is well known, gastro-intestinal nematodes which wander in the final host enter blood vessels or lymph channels after penetrating the skin or the intestinal wall. Then carried passively to the right heart and to the lungs, they free themselves from the capillaries in the lungs and make their way via the alveoli, bronchioles, bronchi, and trachea to the back of the mouth where, caught in the swallowing movements of the host, they become re-routed down the food tube. Skin penetrators like hookworm and *Nippostrongylus muris* have been employed in standardized experiments to establish "normal" infections by using subcutaneous injections to introduce the larvae into the host (Otto and Kerr, 1939; Watt et al. 1943). That *Ascaris* can be shown to demonstrate the lung-wandering stage by introducing infective eggs into the mouth of a guinea pig is a common laboratory exercise.

The inability of *Haemonchus* as a skin penetrator has been recognized since Veglia's (1916) experiments. If *Haemonchus* possessed the ability to wander after parenteral injection, it must be assumed the species would demonstrate this when the opportunity was repeatedly presented. The fact that none of 26 sheep and lambs, and one calf, injected subcutaneously with a total of $1\frac{1}{2}$ million larvae, showed any sign of infection established in the abomasum, would appear to be adequate evidence of inability to classify *Haemonchus* as a wandering form.

Of 6 animals in our series injected intraperitoneally with a total of over 3 million larvae, 2 examined at autopsy 3 and 6 days later, and 4 examined by fecal examinations for periods up to 11 weeks after the injections, showed no signs of *Haemonchus* reaching the abomasum. Two of these must be considered especially severe tests. In one case 1,300,000 were injected in a single dose. In the other, there were four successive weekly injections totalling nearly a half-million larvae. In neither of these was there found any sign whatever that larvae reached the abomasum. The former was negative at autopsy 3 days after injection, and the latter negative for a period of 11 weeks with 1 to 4 fecal examinations weekly.

Fertile *Haemonchus* infections were later established by the normal route in 31

of the 32 above ovine hosts. The 32nd was negative at autopsy when killed 7 weeks after injection. The influence of the preliminary parenteral larval injections is elsewhere noted (Stoll, 1942).

The fact that 5 other "intraperitoneally" injected sheep (one examined at autopsy 12 days later, 4 by fecal examinations only) did show establishment of worms is less readily explained as a demonstration of wandering of the larvae than of direct penetration of some one of the four stomachs of the sheep, presumably the rumen, by the inoculating needle. Ancillary data tend to confirm this presumption. In H52, which came to autopsy 12 days after injection, an area in the rumen wall, indicative of earlier trauma, was observed. H96 showed a high egg count at the usual interval appropriate for a mouth infection, and there was evidence at the time of injection that apparently the rumen had been entered. H97, injected four times at weekly intervals, showed egg counts appropriately explainable as experimental error in entering the rumen on the 1st and 4th injections, and no appropriate egg count rise for the 2nd and 3rd injections. Concerning the other two positives, H33 and H37, which were the first two animals injected, no further evaluation can be given due to their subsidiary use in another experiment, and their inadequate examination record.

For any one who has attempted intraperitoneal injections through the taut abdominal wall of sheep with materials whose fate, if reaching the lumen of a hollow organ, can be demonstrated there, the fact that penetration of the rumen should occasionally have occurred will not seem strange. McCoy (1935) has noted that injection of rats with live *Trichinella spiralis* larvae occasionally caused infections to become established, presumably because the needle accidentally penetrated the intestine of the rat.

It may be emphasized that if *Haemonchus* is a typical wanderer, even one animal injected parenterally and not showing subsequent infection would outweigh in significance several which did.

These findings are not in disagreement with the mild and transient degree of invasion of the glandular openings in the stomach wall by larvae under conditions of normal infection.

SUMMARY

Infective *Haemonchus* larvae, rendered axenic and injected subcutaneously into 26 sheep and 1 calf, failed to show establishment of infection in the abomasum. A similar failure to establish abomasal infections occurred after intraperitoneal injection of 6 sheep. The hosts ranged in age from 2 weeks to 13 months, the doses from 24,000 to 1,300,000. The series constitutes adequate evidence that this nematode is not a wandering form. Five additional "intraperitoneally" injected sheep later showed abomasal infection, interpreted as accidental injection of larvae directly into the rumen.

Lodgment of *Haemonchus* larvae in the gastric pits during the first parasitic stage following the normal oral route of infection is demonstrable but is not regarded as evidence of "wandering" in the usual sense.

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INCREASED INFECTIVITY OF THE EGGS OF THE DWARF TAPE-
WORM (*HYMENOLEPIS NANA* VAR. *FRATERNAL*) FOLLOW-
ING STORAGE IN HOST FECES¹

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In all previous quantitative work on *H. nana* var. *fraterna*, the eggs for infections were isolated from fecal pellets passed recently by infected animals. Shorb (1933) found that the percentage development of these eggs in rats and mice was exceedingly low, ranging from 0.0002 to 0.4 in rats, and from 0.1 to 1.0 in mice. However, Hunninen (1935) in his studies on mice reported much higher percentages of development, with that of cysticercoids ranging from 1.6 to 13.2, with an average of 4.1, and of adult worms from 1.0 to 8.0, with an average of 2.8 per cent. Some of Hearin's (1941) experiments with mice showed about a 3.4 per cent development of cysticercoids, and in a few preliminary experiments of the writer, approximately 4.3 per cent of the eggs administered to mice were observed later to have developed into cysticercoids. Since any practical method for increasing the percentage development of eggs would prove valuable in experimental work, it seemed worthwhile to consider this problem.

Shorb (l.c.) found that eggs isolated from the pellets and stored in tap water rapidly lost their infectivity for mice after three days and that none was infective after 10 days. It was on the basis of these results that he considered the eggs to be most infective immediately after passing from the host. Since water is an unnatural medium for the storage of the eggs, it seemed of interest to test the effect on their viability of storage in the fecal pellets.

Eight experiments were performed at various intervals. The first four of these involved the strain of tapeworm obtained from mice bought from a local dealer which will be designated as the M₁ strain. Another strain which will be called the M₂ strain was obtained from mice of a rural dealer (Larsh, 1943). The experimental procedure followed in the use of each strain was similar. Fecal pellets were collected from a large group of infected mice, and the material divided into two portions. One portion supplied eggs for infecting mice immediately, the other portion was stored for 48-72 hours at room temperature in 50-cc centrifuge tubes filled with tap water. Then eggs isolated from this material were used to infect a similar group of mice of the same age. Ninety-three hours after infection, the animals of both groups were sacrificed and a count made of the number of cysticercoids present. The results of the first four experiments with the M₁ strain are tabulated in Table 1; those of the other four experiments with the M₂ strain are shown in Table 2.

In the experiments with the M₁ strain, Table 1, the mice receiving eggs that had been stored three days in feces had about twice as many cysticercoids (8.8-12.8 per cent development) as those receiving eggs from fresh feces (4.5-6.1 per cent devel-

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opment). In the experiments with the M_2 strain, Table 2, the storage in feces likewise appeared to increase development although the percentage of development was not quite twice that with the fresh eggs, being respectively 4.4–6.4 per cent and 2.8–4.1 per cent. These two strains showed constant differences in their host infectivity. A discussion of their relations is reserved for another paper.

TABLE 1.—Showing the percentage development of cysticercoids in mice two and one-half months old infected with unstored and stored eggs of the M_1 strain

Experiment No.	No. of mice	Egg dose	Average No. of cysticercoids	Standard deviation of individual measurements	Percentage development
A. Eggs isolated from fresh feces					
1	10	900	42.9	6.5	4.8
2	5	1500	67.0	5.0	4.5
3	10	2000	121.3	20.5	6.0
4	5	3000	182.8	10.8	6.1
B. Eggs stored from 48 to 72 hours					
1	10	900	79.0	12.4	8.8
2	5	1500	163.2	11.7	10.9
3	10	2000	255.9	37.4	12.8
4	5	3000	374.8	19.9	12.5

SUMMARY

Eggs of *Hymenolepis nana* var. *fraterna* isolated from fecal pellets that had been stored in an aqueous medium at room temperature for 48–72 hours showed a greatly increased percentage development of cysticercoids in mice compared with eggs from fresh feces. No explanation is offered to account for this increase in infectivity, but it may be an indication of a natural incubation period heretofore not considered.

TABLE 2.—Showing the percentage development of cysticercoids in mice two and one-half months old infected with unstored and stored eggs of the M_2 strain

Experiment No.	No. of mice	Egg dose	Average No. of cysticercoids	Standard deviation of individual measurements	Percentage development
A. Eggs isolated from fresh feces					
5	10	1200	34.4	13.1	2.8
6	5	1500	53.2	8.8	3.5
7	10	2000	78.5	7.4	3.9
8	5	3000	121.8	5.2	4.1
B. Eggs stored from 48 to 72 hours					
5	10	1200	52.6	14.0	4.4
6	5	1500	78.8	10.5	5.3
7	10	2000	127.7	9.0	6.4
8	5	3000	180.0	7.4	6.0

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A NEW ECTOPARASITE (ACARINA: CHEYLETIDAE)
FROM DOMESTIC RABBITS

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During the past six years the Los Angeles Wildlife Disease Research Station, U. S. Fish and Wildlife Service, has conducted a careful search for ectoparasites from more than 3,000 domestic rabbits, *Oryctolagus cuniculus*, that have been brought in for observation. All of these came from rabbitries in California, more than half of them from southern California. Because of hutch construction and approved practices of rabbit husbandry, very few ectoparasites are ever found infesting rabbits raised in commercial rabbitries of southern California.

On December 4, 1942, a three-year-old, New Zealand White, male rabbit was presented to the Station by a nearby rabbit breeder for diagnosis and treatment. Approximately three months before this animal had been placed in a recently acquired, secondhand rabbit hutch. This hutch was not sterilized or disinfected before being used. The seller of the hutch claimed it had been idle for two months.

The first indication of any disease was a fine, powdery, meal-like scruff or dandruff which the owner noted in the fur. This spot was located on the dorsal midline approximately five centimeters behind the shoulder blades. The infested spot was roughly circular in shape and approximately five centimeters in diameter. Much of the under fur in this spot had loosened and fallen out. The guard hairs were less in number than in adjoining areas. A very fine, gray-white, slightly oily scale covered this area. This scale could easily convey to the clinician the impression of the early stages of favus. The skin beneath the scale deposits was red, tender to the touch and slightly eroded. No crusts were formed, neither were there any scratch wounds to indicate the rabbit had attempted to alleviate a local irritation. No other affected areas were found although the body and extremities were carefully examined. Other than the infested area the rabbit appeared normal in every respect. Clinical examinations of the scale, skin scrapings and fur from the affected area failed to demonstrate the presence of any fungi. These same materials did, however, reveal the presence of a mite. It was quite evident that this mite was carrying on its complete life cycle on the body of its host because adults, nymphs, larvae and eggs were found.

From this evidence and the following analysis of these mites they undoubtedly represent a group, heretofore unknown to science, of true mammalian ectoparasites. It is concluded here a new genus, of the family CHEYLETIDAE, is necessary to hold this interesting parasite.

Family Cheyletidae

Ewingella n. g.

Very small mites, body slightly longer than broad, lightly compressed dorso-ventrally; dorsal plate in anterior half only; legs short, all uniform in length and structure; coxae narrow, paired

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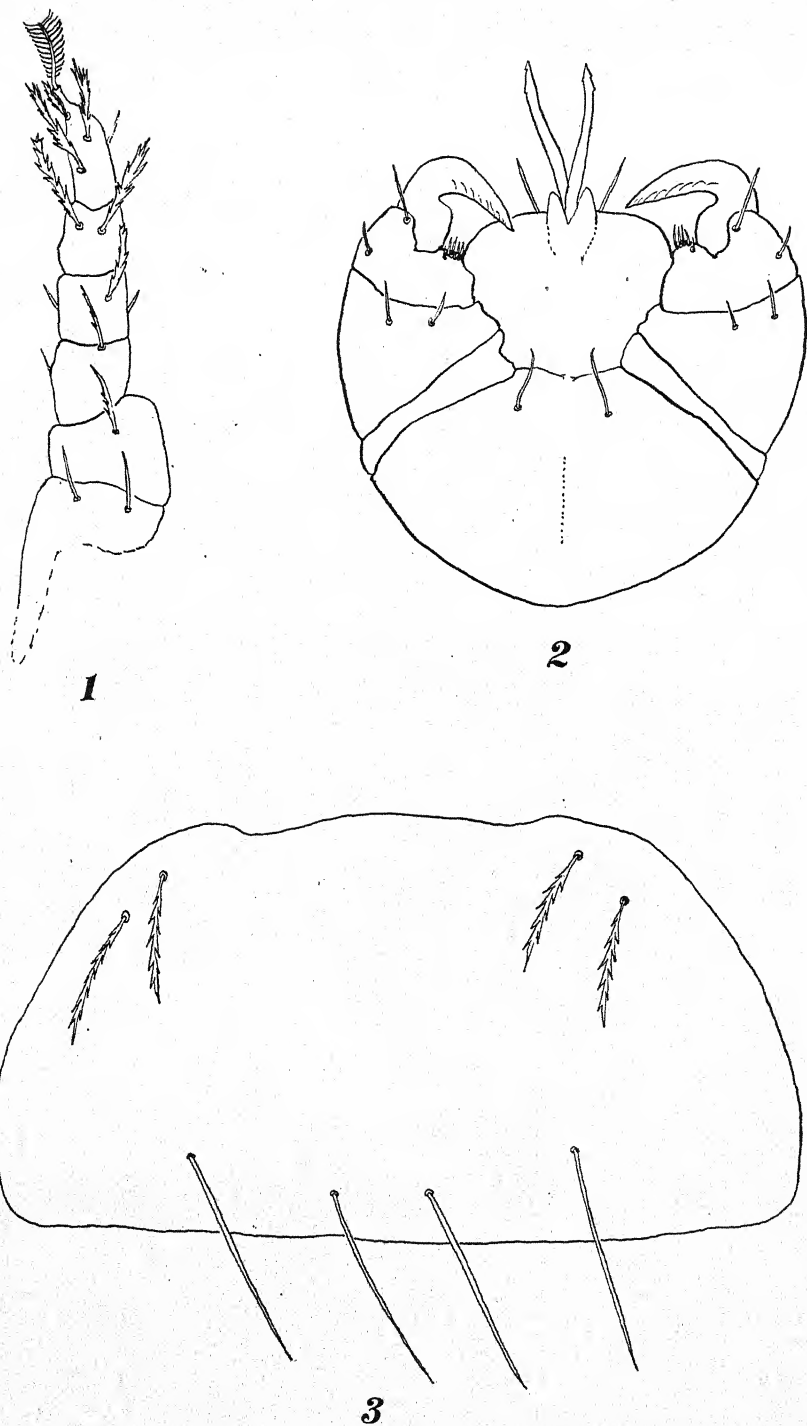


FIG. 1. *Ewingella americana* n. sp., leg III, holotype female.
 FIG. 2. *Ewingella americana* n. sp., capitulum, ventral, holotype female.
 FIG. 3. *Ewingella americana* n. sp., dorsal plate, holotype female.

on a side I with II, III with IV, not joining any of those on the opposite side; chelicerae stylete, retractile; palpi very large, thick, arm-like, the last segment armed with a prominent, falcate claw, with minute opposing thumb; integument of entire body finely striated; setae simple to plumose; tarsi with single claw, all with a double pectinate appendage, analgenital aperture terminal; a dorsal, pseudo-division between cephalothorax and abdomen.

Genotype: Ewingella americana n. sp.

This new genus is nearest to *Cheyletiella*. It differs in the much broader palpi, type of tarsal claw, and, among other things, the single dorsal plate. The genus bears the name of Dr. H. E. Ewing, our foremost acarologist.

Ewingella americana n. sp.

(Figs. 1-3)

Holotype female:

Capitulum: palpi very thick at bases, armlike, opposing each other, terminal claws formidable, falcate, thumb minute, with three to four subequal setae, segment II with long, dorsal, plumose setae, segment III with shorter setae; hypostome and epistome fused, broadly cone-shaped, with fluted lateral trocheal openings; chelicerae stylete; basi capitulum broad, with two upper, sub-median, short setae.

Body Dorsum: slightly broader in anterior half; dorsal plate rectangular, widest posteriorly, with two plumose setae in each antero-lateral angle, four simple setae on posterior margin, surface finely pitted; lateral to dorsal plate two long plumose setae, also one on each shoulder just off dorsal plate; a fold in integument between cephalothorax and abdomen; posterior half of dorsum with two sub-median simple setae just below dorsal plate, two long plumose setae upper and lower on lateral margins, with few small setae in between, also around anal aperture; body terminates in two long bristles; venter with numerous small setae, of which only two in lower lateral angles are plumose; anal-genital aperture continued dorsally, vulva anterior to anus; coxae in pairs, somewhat radially arranged; legs uniform, with scattered plumose setae, as well as plain setae, taper slightly to tarsi, claws double pectinate as a broken feather on a short, non-segmented pedicel.

Holotype: one female on *Oryctolagus cuniculus*, Los Angeles Wildlife Disease Research Station, U. S. Fish and Wildlife Service, Los Angeles, California. Deposited in the Allan Hancock Foundation, University of Southern California, Los Angeles, California.

Paratypes: 12 females, collected and deposited as above.

Members of the mite family CHEYLETIDAE are for the most part predatory in nature, preying upon other mites and small insects, either attacking them directly or indirectly by feeding on their eggs. There are a few groups within this family which apparently lead a parasitic existence. One such group recently received a fine analysis by Ewing (1938). Unfortunately *Cheyletiella canadensis* Banks, 1909, a supposedly parasitic form, is little known, and from all indications the only representative from North America. As Ewing (ibid) has pointed out, much work remains in the study of cheyletid mites, particularly from North America. European workers, i.e., Oudemans, have been far in advance in regards to work completed on these important mites. Banks (1915, p. 28) records an interesting European cheyletid record. "*Cheyletus parasitivorax* Michial uses the rabbit's fur as a hunting forest, where it destroys the *Listrophorus* mites which occur on the hairs of the rabbits." This is typical for the majority of cheyletids, other predatory forms are to be found almost everywhere, even on old agar culture plates, in nests of many birds, rats and mice, both native and domestic.

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RESEARCH NOTES

OBSERVATIONS ON A TRICHOMONAD FROM THE INTESTINE OF MAN

In the intestine of man trichomonad flagellates which possess five anterior flagella frequently occur (Wenrich, 1931; Arch. Soc. Biol. Montevideo, Suppl.: 1185-1204), and flagellates with four anterior flagella but otherwise identical have been reported to exist. The discrepancy in accounts of the number of anterior flagella may be the result of inaccurate observation or alteration of normal structure in the specimens examined, or may be due to genetic differentiation or factors in development giving real differences. The opinion is growing that the common trichomonad flagellates of the intestine of man belong to one species, which may be named *Pentatrachomonas hominis* (Davaine), as suggested by Wenrich (1931), or *Trichomonas hominis* Davaine, as used by Wenrich (1942, J. Parasitol. 28 Suppl.: 12). If this opinion is accepted, either the number of anterior flagella must be considered a variable characteristic, or reports of numbers other than five must be rejected. It is evidently on the basis of the former consideration that Wenrich (1942) stated that in *Trichomonas hominis* there are four or five anterior flagella. It is probable, in my opinion, that the complement of anterior flagella is a definite and stable characteristic in these trichomonads, as in other flagellates; if a four-flagellate form exists it deserves specific differentiation. This account will deal with a five-flagellate form found in a stool specimen sent into the laboratory by a person in California, and maintained in culture for eight months.

It is important that emphasis be given to the fact that the five anterior flagella are not in one group. It has been indicated by Kofoid and Swezy (1923, Univ. Calif. Publ. Zool. 20: 373-390)

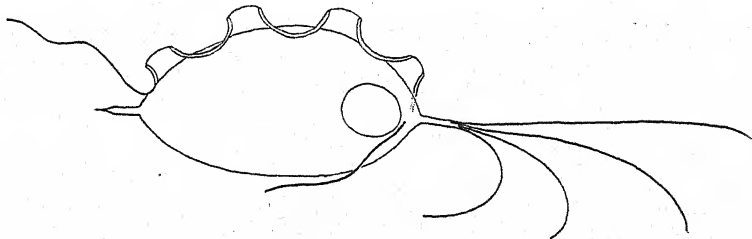


FIG. 1. *Pentatrachomonas hominis* (Davaine). Diagram of outline structure as demonstrated by darkfield illumination. $\times 3000$.

and by Wenrich (1931) that there is a group of four flagella and an independent flagellum. By the use of darkfield illumination I have determined this number and arrangement of flagella in more than fifty living flagellates, which had been mounted under paraffin-sealed coverslips and left to become sufficiently slow in their movements. (Many were still very active after 24 hours at room temperature, and a few were alive after five days.) The group of four flagella originates at the anterior end of the body, its base appearing in darkfield as a columnar extension that shows only the two edges. After a length of 1 to 2 μ this column divides into four flagella. The length of the flagella in a group varies. Whatever the length of the body, which ranged in this material from 8 to 15 μ , the longest flagella measure 15 to 18 μ ; the shortest are sometimes 8 or 10 μ . At one time in their course of movement they are turned back together against the ventral side of the body, and are at rest for an instant. The group is then thrown quickly across from the ventral to the dorsal side and rapidly forward; immediately, one following another, the flagella swing posteriorly to a position against the ventral side of the body. In this movement each flagellum becomes curved; when extended forward its concavity is toward the ventral side. The above description is of the flagellar movement seen in many specimens confined in one position and much less active than normal, but it probably indicates the pattern followed in the very rapid unmodified activity of the flagellate.

The independent flagellum appears by darkfield illumination to originate in a clear bright end about a micron posterior to the beginning of the whip of anterior flagella, on the side of the body opposite to the beginning of the undulating membrane. It is entirely separate from the flagella of the anterior whip, and from its origin is directed posteriorly, in the manner of a trailing flagellum. Its length varies from 6 to 13 μ ; generally it is about half as long as the longest flagella of the anterior whip. It is moved backward and forward, usually in a limited arc, maintaining in general its posteriad direction on the ventral side. At times it is turned forward

or across the body, but it does not for the most part change position more than the free flagellum at the posterior end of the undulating membrane. The activity of the independent flagellum is much more like that of the membrane flagellum than like that of the flagellar whip, as was pointed out by Kofoid and Swezy (1923).

Some writers at the present time place all trichomonads with an undulating membrane into the genus *Trichomonas*, whether the anterior flagella number three, four, or four plus one (Wenrich, 1942). Differences in flagellar organization should be considered indicative of differentiation of groups of trichomonads, as well as of other flagellates. The trichomonad with which this report is concerned possesses a structure, the independent flagellum, which is a new addition, of a unique sort, to the organization that exists in *Trichomonas*. It is my opinion that it warrants generic distinction for the flagellate, and that the name *Pentatrachomonas* should be retained. It will never be known what was the structure of Davaine's flagellate, any more than of O. F. Müller's *Cercaria tenax*, except insofar as we can make plausible identification of their forms with flagellates that can be studied by modern methods. Nevertheless, there is probably as much justification for using the name *Pentatrachomonas hominis* (Davaine) for the common trichomonad of the intestine of man as for adopting *Trichomonas tenax* (O. F. M.) for the flagellate of the mouth of man.—HAROLD KIRBY, Department of Zoology, University of California.

GIARDIA IN THE BLOOD OF A KANGAROO RAT

Various species of *Giardia* have been reported from a great variety of animals, usually inhabiting the duodenum or small intestine. There are very few reports of its occurrence elsewhere in the body of the host. Wenyon (1926, Protozoology) cites a report by Gonder, in 1910, of *Giardia* in blood films from a falcon shot in the Transvaal. Wenyon is of the opinion that this blood film was contaminated with intestinal material. He also cites Fantham's report, in 1919, of a *Giardia* from the blood, as well as the intestine of the South African silver fish. There have been several reports of the related genus *Hexamita* occurring in the blood. Recently Andrews (1941, J. Parasitol. 27 Suppl: 26) has reported it from experimental mice.

In February, 1942, a kangaroo rat (*Dipodomys heermanni*) was trapped on the San Joaquin Experimental Range, Madera County, California, and shipped to the laboratory of the U. S. Fish and Wildlife Service in Los Angeles. The animal was killed with ether. A blood smear was made from the heart and stained with Giemsa's stain. This thin film was a clean smear and showed no evidence of any possible contamination from the intestine. A single trophozoite of *Giardia* was observed in this blood film. It was similar in morphology to *Giardia* observed from the intestine of kangaroo rats.—CARLTON M. HERMAN, California Division of Fish and Game.

COMPARING THE PERCENTAGE DEVELOPMENT OF THE DWARF TAPEWORM, *HYMENOLEPIS NANA* VAR. *FRATERNA*, OBTAINED FROM MICE OF TWO DIFFERENT LOCALITIES

In connection with a recent series of studies on the dwarf tapeworm, *H. nana* var. *fraterna*, in mice, data were obtained which suggest a physiological dissimilarity between two parasites isolated from mice of different localities. The first tapeworms were taken from white mice bought locally. In various experiments, a total of 50 mice, 2½ months old, were given initial infections and the resulting percentage development of cysticercoids ranged from 8.8, when 900 eggs were administered, to 12.8 following a test dose of 2000 eggs.

The first stock infection was lost during the summer of 1941 and another was established with worms from mice sold by a rural dealer. In all of the experiments performed to date, the percentages of cysticercoid development have been strikingly lower than those from similar infections with the first tapeworms. More than 150 mice, 2½ months old, have been tested as controls in various experiments in which infective egg doses varied from 900 to 3500. The range of cysticercoid development was from 4.2 to 6.6 which is about one-half that in mice of this age infected with eggs from the previous stock. Other infections in mice of other ages showed constant differences between these two parasites.

It is important to add that the percentages given for either of these parasites can not be compared directly with those of previous workers, because a new method of fecal storage was used to obtain eggs for infections (Larsh, 1943, J. Parasitol. 29: 417-418). By this procedure, it was demonstrated that the percentage development of eggs into cysticercoids is consistently higher than that obtained with eggs from fresh feces. It is interesting to note that eggs of the second stock used for infections before storage, i.e., isolated from fresh feces, showed about the same percentage developments as those of a parasite obtained earlier from the same source by Hunninen (1935, Am. J. Hyg. 22: 414-443). The average percentage in his study was about

4.1, whereas that of the unstored eggs in the present study was approximately 3.8. In all probability, the parasite used in both cases was of the same line. Heretofore, the differences in percentage development reported by various workers using the mouse strain of *H. nana* in mice have been attributed to personal factors, but perhaps they were due, after all, to slightly different strains of the parasite.

The results given above suggest that the two parasites studied are different physiologically. Just how much they differ can not be ascertained except by direct comparisons. Shorb (1935, Am. J. Hyg. 18: 74-113) demonstrated two distinct physiological strains of this parasite, one in rats, the other in mice. The finding in addition of marked variations in the mouse form would seem to suggest that different strains of this parasite are produced easily and may be quite numerous. In a recent paper (Rappaport, 1943, Am. J. Trop. Med. 23: 343-367) similar findings are reported for *Trichinella spiralis*.—JOHN E. LARSH, JR., *School of Hygiene and Public Health, Johns Hopkins University*.

A NOTE ON THE LIFE CYCLE OF *TAMERLANEA BRAGAI* SANTOS, 1934 (TREMATODA: EUCOTYLIDAE)

Subulina octona, a common land snail in Puerto Rico, has proved to be a satisfactory intermediate host of *Tamerlanea bragai* Santos, 1934, a kidney fluke of the domestic pigeon, *Columba livia domestica*. This trematode was described by Santos from Brazil (Reis and Nobrega, 1936, Doenças das Aves, Sao Paulo, Fig. 222, p. 313) and reported from Puerto Rico by Maldonado and Hoffman (1941, J. Parasitol. 27: 91).

Preliminary observations indicate that the ova of the parasite are ingested by the snail. Mother sporocysts give rise to delicate, spherical daughter sporocysts. The daughter sporocysts give rise to an average of eight to ten tailless cercariae which at due time encyst inside the sporocyst. The cycle in the intermediate host is completed within two months after ingestion of the ova. Pigeons become infected by feeding on the infected snail. The parasite reaches the adult stage in about two weeks after ingestion of the encysted cercaria.

All the cercariae observed possess a well developed acetabulum. This organ, however, atrophies during development in the final host, but occasionally may be retained in a rudimentary form by the adult. It is suggested that because of the presence of an acetabulum in the cercaria and adults, *Tamerlanea bragai* should be excluded from the group of monostomes in which it is classified at present and included under the suborder Distomata.

A detailed study of the development of the parasite in the snail and final host is under way.—JOSÉ F. MALDONADO, *Department of Medical Zoology, School of Tropical Medicine, San Juan, P. R.*

CYSTICERCUS OF *TAENIA TAENIAEFORMIS* WITH TWO STROBILAE

One of seven strobilocercus larvae of *Taenia taeniaeformis* removed from the liver of a muskrat (*Ondatra zibethica*) captured on the University of Michigan campus, April 14, 1942, was abnormal in that two fully developed strobilocerci, 24 mm in length, were attached to a single

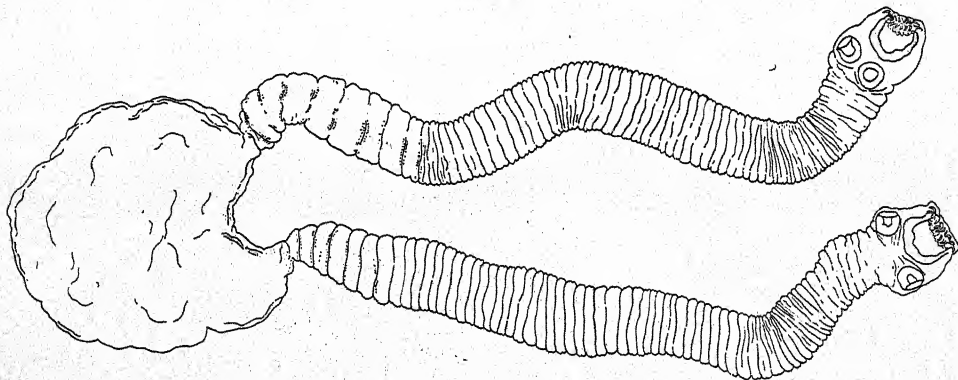


FIG. 1.

bladder, 5×7 mm. (See Fig. 1.) They were entwined around one another and tied into a rather compact knot. These two individuals were of equal size and even larger than some of the other cysticerci removed from the same liver. Each showed normal segmentation, suckers and hooks.

Although anomalies have been reported for larvae of this genus, none seem to have been recorded from North America. Southwell and Kirshner (1937, Ann. Trop. Med. and Parasitol. 31: 37-42) published a description of a polycephalic cestode larva taken from the surface of the spleen of *Mastomys erythroleucus* (a rat) from Sierra Leone, West Africa, which they believed to be *Cysticercus fasciolaris*. In this specimen 12 segmented strobila measuring 20-22 mm in length arose from a single bladder (10 mm in diameter). Dollfus (1938, Ann. Parasitol. 16: 133-141) has described a larva identified as *Taenia taeniaeformis* possessing a single bladder (7×13 mm) with 6 strobila, 10-12 mm in length, attached. The latter polycephalic anomaly was dissected from a white mouse from the French Cameroons, West Africa. More recently Lent (1942, Rev. Brasil Biol. 2: 197-201) has reported the finding of four cysticerci of *Taenia solium* with two scolices each.

The development of more than one individual from a bladder in a species where normally one occurs, indicates multiple centers of growth in the bladder. Thus it would seem that the developing bladder worm may at times possess germinal properties characteristic of *Multiceps* or *Echinococcus*. This seems to accord with Dew's explanation of the origin and development of daughter cysts in *Echinococcus granulosus*. Dew (1925, Med. J. Australia 2: 496-505) considers that the development of daughter cysts is the result of injury of an original hydatid, this injury being chemical, physical or by growth of structures of the surrounding host tissue into the hydatid cyst. These twinned cysticerci of *Taenia* as well as the polycephalic ones may have arisen as a result of injury or disturbance in the early development of their germinal layers. In the present example twinning has gone to completion whereas in tri-radiate, tetra-radiate or penta-radiate worms (Foster, 1915, J. Parasitol. 2: 7-20; Cram, 1926, J. Parasitol. 14: 54-55; Dobrovolsky & Dobrovolsky, 1935, Tr. Am. Micr. Soc. 54: 22-27) a similar condition exists but complete separation of individuals has not been accomplished. It is surprising that more cases have not been reported.—ROBERT E. KUNTZ, Department of Zoology, University of Michigan, Ann Arbor, Michigan.

ADDITIONAL RECORDS OF HUMAN INTESTINAL MYIASIS CAUSED BY *ERISTALIS*

Swartzwelder and Cali, 1942 (Am. J. Trop. Med. 22: 159-163), summarized the reports of human myiasis caused by larvae of syrphid flies. Of 22 cases in the literature, including one reported by these authors, two were ascribed to the genus *Syrphus* and one to *Helophilus pendulus*. All the others presumably belonged to the genus *Eristalis*, or possibly *Helophilus*.

Three hitherto unreported cases of human infection with rat-tailed maggots of the genus *Eristalis* have come to my attention, as follows: (1) a full-grown *Eristalis* larva, the body portion 25 mm long, passed by a child 4 years of age at Tomball, Texas, on July 15, 1941; (2) an *Eristalis* pupa with body 14 mm long passed by a boy 6 years of age at Houston, Texas, on August 17, 1939; and (3) an *Eristalis* larva 18 mm long passed by a child 5 years of age at Houston, Texas, on August 11, 1943.

In each case a single specimen was passed in the feces alive, but failed to continue development when placed in water with decaying vegetation. No additional specimens were passed when anthelmintics or purges were given. In the first case mentioned above vague symptoms of abdominal discomfort were reported, but in the other two cases there were no symptoms referable to the infection.—ASA C. CHANDLER, Rice Institute, Houston, Texas.

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	WILLARD H. WRIGHT		1942-

* Deceased.

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* Deceased.

† Beginning in 1942, the Chairman, Editorial Committee, became ex officio member of Council.

List of Meeting Places

1925 Kansas City	1931 New Orleans	1938 Richmond
1926 Philadelphia	1932 Atlantic City	1939 Columbus
1927 Nashville	1933 Boston	1940 Philadelphia
1928 New York	1934 Pittsburgh	1941 Dallas
1929 Des Moines	1935 St. Louis	1942 (New York, cancelled)
1930 Cleveland	1936 Atlantic City	1943 (No meeting)
	1937 Indianapolis	

AMERICAN SOCIETY OF PARASITOLOGISTS

CONSTITUTION

(Including revisions of Dec. 30, 1931, Dec. 28, 1932, Dec. 29, 1933, Dec. 31, 1940 and Dec. 30, 1941.)

NAME AND OBJECT

The name of the society is the American Society of Parasitologists.

The object of the society is the association of workers in the field of Parasitology for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as will tend to the advancement of parasitological teaching and investigation in this country.

MEMBERSHIP

The members of the society shall be of two classes, active and foreign honorary.

Any person interested in parasitology may be a candidate for active membership.

Any foreign scientist who has made eminent contribution to Parasitology may be eligible for honorary membership.

Candidates for membership shall be elected by the Council.

OFFICERS

The officers of the society shall be a President and a Vice-President, who shall be elected for one year; a Secretary and a Treasurer, who shall be elected for two years; and members at large of the Council.

The Council shall consist of the President, the Vice-President, the Secretary, the Treasurer, the Chairman of the Editorial Committee, and eight members elected by ballot from the society at large, two for four years, two for three years, two for two years and two for one year. After the first year two members at large of the Council shall be elected each year to serve four years.

If any vacancy occurs among the officers, the Council is authorized to appoint a member to fill out the unexpired portion of the current year.

The routine business of the society shall be administered by the Council.

Five shall constitute a quorum of the Council.

DUES

The dues, to include subscription to the JOURNAL OF PARASITOLOGY, shall be four dollars the year for active members, unless changed by vote of the society.

MEETINGS

There shall be an annual meeting and such other scientific or business meetings as the Council shall determine.

During the annual meeting a business meeting will be held for the election of officers for the ensuing year and for the transaction of other business.

JOURNAL OF PARASITOLOGY

This Journal, the property of the society, is its official organ. Responsibility for its conduct shall rest with the Council which shall select the editorial staff and set the price of subscription.

ENDOWMENT FUND

Provision is made for the establishment of a permanent endowment fund, the principal of which may be expended only by a three-fourths vote of all members of the Council and approval by a three-fourths vote of the members of the society present at a regular meeting. The Council shall be entrusted with the maintenance of the fund, and the use of the income therefrom.

AMENDMENT

On recommendation of a two-thirds vote of the Council, the constitution may be amended by a two-thirds vote of the members present at any regular business meeting of the society, provided that at least 30 days' notice has been given to the membership of the proposed amendment.

BY-LAWS

MEMBERSHIP

1. *Election of Members.* An affirmative vote of all Council members present at a meeting shall be necessary for the election of candidates for membership. If the vote is taken by mail ballot, an affirmative vote of all members of the Council replying within thirty days shall be required.

2. *Members in Good Standing.* Members in good standing are those whose current dues are paid.

3. *Delinquent Members.* The JOURNAL OF PARASITOLOGY shall not be sent to members in arrears, and members in arrears for the current year and two previous years shall be dropped from the roll of the society at the end of the current year.

4. *Reinstatement of Delinquent Members.* Members dropped for non-payment of dues, or who have resigned, may be reinstated by the payment of all dues in arrears. Otherwise, the applicant must apply for election as a new member.

5. *Non-subscribing Members.* Where a second membership in the society is taken in the same immediate family, the second member may join upon the payment of annual dues of one dollar, but without receiving the JOURNAL OF PARASITOLOGY.

6. *Honorary Life Members.* Upon unanimous vote, the Council may recommend that the society confer honorary life membership upon distinguished American parasitologists over sixty years of age. Honorary Life Members shall enjoy full membership privileges and shall be exempted from the payment of dues. The number of Honorary Life Members at any one time shall be limited to five.

7. *Foreign Honorary Members.* Upon unanimous vote, the Council at its annual meeting may elect Foreign Honorary Members. No two men from the same country shall be elected in the same year. The number of Foreign Honorary Members at any one time shall be limited to

twelve. Foreign Honorary Members may receive the JOURNAL OF PARASITOLOGY upon payment of membership dues.

PRESENTATION OF PAPERS AT ANNUAL MEETINGS

1. Except for invited papers, and papers coming to the American Society of Parasitologists program through joint sessions with another society or section, all persons presenting papers must be members of the society in good standing, or must be introduced by a member in good standing.

2. Each member of the society in good standing may be allotted not more than fifteen minutes of program time, to be used in person or by a non-member introduced by him. If the program is crowded, the maximal allotment of time may be reduced to ten minutes.

3. Papers offered for presentation too late to be included on the printed program may be presented at the conclusion of any of the scientific sessions provided that the presiding officer obtains the consent of the members present.

OFFICERS

1. The Council shall act as a nominating committee and at the annual meeting shall submit to the society at least one nominee for each office to be filled. The Secretary shall invite the members of the society to submit nominations for consideration by the Council.

2. The Treasurer of the society shall act also as Treasurer for the JOURNAL OF PARASITOLOGY and shall be bonded for the sum of two thousand dollars.

3. A sum of fifty dollars shall be allotted annually to the Secretary, to the Treasurer, and to the Chairman of the Editorial Committee for attendance at the annual meeting.

MANAGEMENT OF THE JOURNAL OF PARASITOLOGY

1. The JOURNAL OF PARASITOLOGY, as the official organ of the society, shall be managed by an Editorial Committee appointed by the Council for a five-year period, and shall consist of one Protozoologist, one Helminthologist, and one Entomologist, one of whom shall be appointed by the Council to act as Chairman.

2. The Editorial Committee shall be assisted by an Editorial Board consisting of twelve members appointed by the Council for a four-year period in such a way that three members will retire and three new members shall be elected each year. The Editorial Board shall consist of two Entomologists, four Protozoologists, and six Helminthologists to be elected on the basis of attainment, interest in the society and geographical location.

3. The price of the JOURNAL OF PARASITOLOGY shall be five dollars per volume, except to members of the American Society of Parasitologists who shall receive it as a membership privilege included in the annual dues of four dollars.

ENDOWMENT FUND

1. Council shall select a Custodian of the Endowment Fund and two associates to whom it may delegate responsibility for management of the fund. The Custodian shall make an annual accounting to Council and such other reports as Council may request. The approval of two of the three custodians shall be necessary for the purchase, sale or exchange of securities. One of the three custodians shall be the Treasurer of the society and his signature shall be required on all vouchers of expenditure from the fund.

ADDITIONS AND AMENDMENTS

1. Additional by-laws may be created by a two-thirds vote of Council members present at a meeting, or by an affirmative vote of nine Council members in a ballot conducted by mail. By the same procedure existing by-laws may be repealed, amended or suspended.

AMERICAN SOCIETY OF PARASITOLOGISTS

Notice of Election of Officers

The Council of the American Society of Parasitologists, having voted to suspend annual meetings of the Society until travel restrictions are lifted, has recommended that President Henry E. Meleney appoint a Special Committee to select nominees for the offices of the Society for the year 1944. President Meleney has named the following members to this Committee: H. W. Stunkard (Chairman), F. C. Bishopp, and D. H. Wenrich. A ballot bearing the names of those persons nominated for office by the Special Committee is being sent to every member of the Society in good standing for his vote. On this ballot space will be available to permit members, if they wish, to vote for other persons besides those recommended by the Special Committee. It is urged that every member, whether or not he endorses the Special Committee's nominees, participate in the vote, returning at once the ballot, which is already stamped and addressed, to the Secretary.

JAMES T. CULBERTSON,
Secretary.

INDEX FOR VOLUME 29, NOS. 1-6

Acanthocephala: A redescription of <i>Polymorphus obtusus</i> Van Cleave, 1918	289
Acanthocephala of genus <i>Corynosoma</i> from California sea-lion	102
<i>Aedes aegypti</i> , hatching of eggs of, under sterile conditions	324
<i>Ambystoma maculatum</i> (Shaw), a trematode from urinary bladder of	226
AMERICAN SOCIETY OF PARASITOLOGISTS:	
Constitution	431
Minutes, 31st Council Meeting, New York City, January 9, 1943	236
Notice of election of officers	434
Notice of postponement of annual meeting of the society	366
Officers	429
Anaerobiosis and cholesterol as growth requirements of <i>Endamoeba histolytica</i>	278
Anatomy, segmental, of tapeworm, <i>Mesocestoides variabilis</i> Mueller, 1928, from opossum	217
ANNEREAUX, R. F. <i>Opecoelina pharynmagna</i> n. sp. (Trematoda) from the China rockfish	155
<i>Anopheles albimanus</i> Wied., use of sea water in control of	356
<i>Anopheles quadrimaculatus</i> , rate of growth of, in relation to temperature	107
<i>Anopheles walkeri</i> , studies on biology of	117
Anopheline mosquito larvae, tray for collecting	229
Anthelmintics, bioassay of, in <i>Nippostrongylus muris</i> infection in albino rats	42
Anthelmintics, bioassay technique for	48
<i>Apharyngostrigea cornu</i> (Zeder), bird fluke, notes on genital system of	270
<i>Aspidogaster conchicola</i> , trematode, maintenance outside body of its natural host	127
AUGUSTSON, G. F. (see Vail and Augustson)	419
<i>Australorbis glabratus</i> , snail intermediate host of <i>Schistosoma mansoni</i> , note on life cycle of	231
Avian coccidia, selective action of sulfaguandine on	362
Bacterium (<i>Corynebacterium lipoptenae</i>), associated with louse fly, <i>Lipoptena depressa</i> Say	80
Bats in United States, new locality for <i>Trypanosoma vespertilionis</i> (= <i>T. cruzi</i> ?) in	363
BEAVER, PAUL C. A tray for collecting anopheline mosquito larvae	229
BEAVER, PAUL C. Studies on <i>Protechinostoma mucronisertulatum</i> , n. g., n. n. (<i>Psilostomum reflexae</i> Feldman, 1941), a trematode (Echinostomidae) from the sora rail	65
Bedbug (<i>Cimex lectularius</i>), further attempts to transmit <i>Pasteurella tularensis</i> by	395
BEQUAERT, J. Notes on Hippoboscidae. 16. Hippoboscidae from southern Brasil. With the description of a new species of <i>Lynchia</i>	131
Bioassay of anthelmintics in <i>Nippostrongylus muris</i> infection in albino rats	42
Bioassay technique for anthelmintics	48
Biology of <i>Anopheles walkeri</i>	117
Biology of <i>Ornithodoros hermsi</i>	33
Biology of the argasid tick, <i>Ornithodoros nicolleti</i> Mooser	393
Birds from southwestern United States, blood parasites in	187
Birds trapped in Athens, Georgia, blood protozoa of	260
BLISS, C. I. (see Whitlock and Bliss)	48
BOOKS AND MONOGRAPHS RECEIVED	426
Branchiobdellids, report on a collection of	100
Brasil, Hippoboscidae from southern	131
BRODY, ARTHUR L. AND EDWARD F. KNIPLING. Can larvae of <i>Cochliomyia americana</i> C. and <i>P.</i> mature in carcasses?	59
BROOKS, F. G. Larval trematodes of northwest Iowa. I. Nine new xiphidiocercariae	330
BROOKS, F. G. Larval trematodes of northwest Iowa. II. Four new strigeids	340
BROOKS, F. G. Larval trematodes of northwest Iowa. III. A new collarless echinostome cercaria	347
BYRD, ELON E. AND JAMES W. WARD. Notes on the genital system of the bird fluke, <i>Apharyngostrigea cornu</i> (Zeder)	270
BYRD, ELON E. AND JAMES W. WARD. Observations on the segmental anatomy of the tapeworm, <i>Mesocestoides variabilis</i> Mueller, 1928, from the opossum	217
CABLE, R. M. (see Hunninen and Cable)	71
California, food habits and intensity of coccidian infection in native valley quail in	206
California sea-lion, Acanthocephala of genus <i>Corynosoma</i> from	102
Canine strongyloidiasis in Texas, case of	157

Carcasses, can larvae of <i>Cochliomyia americana</i> C. and P. mature in?	59
<i>Cercaria stagnicola</i> Talbot, 1936, schistosome, development of sporocysts of	164
Cercariae, new from northwest Iowa	330; 340; 347
Cercariae, two new large-tailed (Psilostomidae) from northern Michigan	182
CHANDLER, ASA C. A case of canine strongyloidiasis in Texas	157
CHANDLER, ASA C. Additional records of human intestinal myiasis caused by <i>Eristalis</i>	425
CHANDLER, ASA C. A redescription of <i>Contracaecum habena</i> (Linton, 1900) Linton, 1934	156
CHATTIN, JOHN E. (see Herman, Chattin and Saarni)	206
CHEATUM, E. L. (see Goble and Cheatum)	230
Children, in Ohio institution, prevalence of pinworm infection in	298
Cholesterol and anaerobiosis as growth requirements of <i>Endamoeba histolytica</i>	278
<i>Cimex lectularius</i> , bedbug, further attempts to transmit <i>Pasteurella tularensis</i> by	395
Coccidia, avian, selective action of sulfaguanidine on	362
Coccidia, life cycle of four intestinal, of domestic rabbit	10
Coccidian infection of quail in California, food habits and intensity of	206
<i>Cochliomyia americana</i> larvae, can they mature in carcasses?	59
Cockroaches, a nematode (<i>Thelastoma icemi</i>) from	404
Colorado trematode studies. I. A further contribution to the life history of <i>Crepidostomum farionis</i>	379
<i>Contracaecum habena</i> (Linton, 1900) Linton, 1934, a redescription of	156
Control of <i>Anopheles albimanus</i> , use of sea water in	356
Control of nodular worm disease, overwinter loss of nodular worm larvae from a sheep pasture and its bearing on	263
CORT, W. W. In memoriam. Winfield Carey Sweet (1891-1942)	364
CORT, W. W. AND LOUIS OLIVIER. The development of the larval stages of <i>Plagiorchis muris</i> Tanabe, 1922, in the first intermediate host	81
CORT, W. W. AND LOUIS OLIVIER. The development of the sporocysts of a schistosome, <i>Cercaria stagnicola</i> Talbot, 1936	164
<i>Costia pyriformis</i> , new polymastigine flagellate parasitic on trout	385
CRAWFORD, WILEY W. Colorado trematode studies. I. A further contribution to the life history of <i>Crepidostomum farionis</i> (Müller)	379
<i>Crepidostomum farionis</i> , trematode, further contribution to life history of, in Colorado	379
<i>Critidia fasciculata</i> Léger, 1902, flagellate parasite of mosquitoes	196
CULBERTSON, JAMES T. Natural transmission of immunity against <i>Trichinella spiralis</i> from mother rats to their offspring	114
Cultivation in vitro of metacercariae free from microorganisms	319
Cultivation of <i>Histomonas meleagridis</i> , new medium for	353
Cultivation of <i>Nyctotherus cordiformis</i>	292
Cultures, viability of <i>Trypanosoma</i> and <i>Leishmania</i> in	275
Cuterebridae, warble flies, of rodents	311
Cysticercus of <i>Taenia taeniaeformis</i> with two strobilae	424
DALMAT, HERBERT T. A contribution to the knowledge of the rodent warble flies (Cuterebridae)	311
DAVIS, GORDON E. Further attempts to transmit <i>Pasteurella tularensis</i> by the bedbug (<i>Cimex lectularius</i>)	395
DAVIS, GORDON E. Studies on the biology of the argasid tick, <i>Ornithodoros nicolleti</i> Mooser	393
DAVIS, H. S. A new polymastigine flagellate, <i>Costia pyriformis</i> , parasitic on trout	385
Deer, white-tailed, notes on adults of <i>Protostrongylus coburni</i> in lungs of	158
DENCE, WILFORD A. A leech feeding on ligula	299
Description of <i>Phyllodistomum etheostomae</i> Fischthal, 1942 (Trematoda: Gorgoderidae)	7
Description of <i>Stempellia moniezi</i> Jones, 1942, microsporidian parasite (Nosematidae) of cestodes	373
Development of dwarf tapeworm (<i>H. nana fraterna</i>), from mice of two different localities	423
Development of eye flukes of fishes in lenses of frogs, turtles, birds and mammals	136
Development of larval stages of <i>Plagiorchis muris</i> in first intermediate host	81
Development of sporocysts of schistosome, <i>Cercaria stagnicola</i> Talbot, 1936	164
DEVOLT, H. H. A new medium for the cultivation of <i>Histomonas meleagridis</i>	353
<i>Dictyogium chelydrae</i> , new trematode from snapping turtle	143
Digestive gland tubules in snail <i>Stagnicola</i> , modification of, following parasitization	176
DIKMANS, G. (see Price and Dikmans)	233
<i>Dipetalonema arbuda</i> Highby from the porcupine (<i>Erethizon dorsatum</i>), mosquito vectors and larval development of	243

<i>Dipetalonema arbuta</i> n. sp. from the porcupine, <i>Erethizon dorsatum</i>	239
<i>Dirofilaria scapiceps</i> (Leidy, 1886), from snowshoe hare in Minnesota, vectors, transmission, development and incidence of	253
<i>Dispharynx spiralis</i> in pheasants in New York	230
Dogs, efficacy of product from oil of rose geranium for removal of intestinal parasites from ..	151
DONALDSON, ALAN W. The prevalence of pinworm infection in an Ohio institution for children	298
DOUGHERTY, ELLSWORTH C. (see Goble and Dougherty)	397
Echinostome cercaria, new collarless from northwest Iowa	347
Ectoparasite, new (Acarina: Cheyletidae) from domestic rabbits	419
Eggs and preinfective larvae of <i>Haemonchus contortus</i> , survival on grass plots	284
Eggs of <i>Aedes aegypti</i> , hatching of, under sterile conditions	324
Eggs of dwarf tapeworm (<i>H. nana fraterna</i>), increased infectivity of, following storage in host feces	417
<i>Endamoeba histolytica</i> , growth requirements of	278
<i>Erethizon dorsatum</i> , porcupine, <i>Dipetalonema arbuta</i> n. sp. from	239
<i>Erethizon dorsatum</i> , mosquito vectors and larval development of <i>Dipetalonema arbuta</i> Highby from	243
<i>Eristalis</i> , additional records of human intestinal myiasis caused by	425
Eye flukes of fishes, development of, in lenses of frogs, turtles, birds and mammals	136
Feces, increased infectivity of eggs of dwarf tapeworm following storage in	417
FERGUSON, M. S. Development of eye flukes of fishes in the lenses of frogs, turtles, birds and mammals	136
FERGUSON, M. S. Experimental studies on the fish hosts of <i>Posthodiplostomum minimum</i> (Trematoda: Strigeida)	350
FERGUSON, M. S. In vitro cultivation of trematode metacercariae free from microorganisms ..	319
FISCHTHAL, JACOB H. A description of <i>Phyllodistomum etheostomae</i> Fischthal, 1942 (Trematoda: Gorgoderidae), from percid fishes	7
FISCHTHAL, JACOB H. Number of larvae and time required to produce active immunity in rats against <i>Trichinella spiralis</i>	123
Fish, China rock, <i>Opecoelina pharyngmagna</i> n. sp. from	155
Fish hosts of <i>Posthodiplostomum minimum</i> , experimental studies on	350
Fishes, development of eye flukes of, in lenses of frogs, turtles, birds and mammals	136
Fishes, percid, <i>Phyllodistomum etheostomae</i> from	7
Flagellate (<i>Costia pyriformis</i>), parasitic on trout	385
Flagellate parasites of mosquitoes, with special reference to <i>Crithidia fasciculata</i> Léger, 1902 ..	196
Flatworms from oyster-drilling snail, <i>Thais floridana haysae</i> Clench	362
Flea, oriental rat (<i>Xenopsylla cheopis</i>), in Michigan	300
Flies, warble (Cuterebridae), of rodents	311
Flowers as source of mosquitoes	328
Fly (<i>Lipoptena depressa</i> Say), a new bacterium associated with the	80
Food habits and intensity of coccidian infection in quail in California	206
Fowl (<i>Gallus gallus</i>), <i>Sarcocystis rileyi</i> in	300
Fowls, domestic, spleen volume in, in <i>Plasmodium gallinaceum</i> studies	208
<i>Gallus gallus</i> , domestic fowl, <i>Sarcocystis rileyi</i> in	300
Genital system of bird fluke, <i>Apharyngostrigea cornu</i> (Zeder)	270
Georgia, blood protozoa of birds trapped at Athens	260
Georgia, incidence of blood parasites in birds from	153
Geranium, rose, efficacy of product from oil of, for removal of intestinal parasites from dogs ..	151
<i>Giardia</i> in blood of kangaroo rat	423
GOBLE, FRANS C. Notes on the adults of <i>Protostrongylus coburni</i> in the lungs of white-tailed deer	158
GOBLE, FRANS C. AND E. L. CHEATUM. <i>Dispharynx spiralis</i> in golden and ring-necked pheasants in New York	230
GOBLE, FRANS C. AND ELLSWORTH C. DOUGHERTY. Notes on the lungworms (genus <i>Protostrongylus</i>) of varying hares (<i>Lepus americanus</i>) in eastern North America	397
GOODNIGHT, CLARENCE J. Report on a collection of branchiobdellids	100
GRAHAM, EDWARD AND JACOB UHRICH. Animal parasites of the fox squirrel, <i>Sciurus niger rufiventris</i> , in southeastern Kansas	159
Guinea pigs infected with <i>T. spiralis</i> , estimation of histamine in	367
<i>Gyrocoelia milligani</i> Linton, 1927, type of	230

<i>Haemonchus contortus</i> , survival on grass plots of eggs and preinfective larvae	284
<i>Haemonchus</i> , wandering of, in sheep host	407
HAMANN, C. B. Estimation of histamine in the blood and other tissues of rats and guinea pigs infected with <i>Trichinella spiralis</i>	367
Hare, snowshoe, in Minnesota, vectors, transmission, development, and incidence of <i>Dirofilaria scapiceps</i> from	253
Hares (<i>Lepus americanus</i>), lungworms of, in eastern North America	397
Hassall, Albert (1862-1942), portrait and in memoriam	232
HAWKINS, PHILIP A. <i>Sarcocystis rileyi</i> (Stiles, 1893) in the domestic fowl, <i>Gallus gallus</i>	300
HEDRICK, LESLIE R. Two new large-tailed cercariae (<i>Psilostomidae</i>) from Northern Michigan	182
Helminths from robin, with description of new nematode, <i>Porrocaecum brevispiculum</i>	161
HERMAN, CARLTON M. <i>Giardia</i> in the blood of a kangaroo rat	423
HERMAN, CARLTON M., JOHN E. CHATTIN AND ROY W. SAARNI. Food habits and intensity of coccidian infection in native valley quail in California	206
HERMAN, CARLTON M. (see Wood and Herman)	187
HIGHBY, PAUL R. <i>Dipetalonema arbuta</i> n. sp. (Nematoda) from the porcupine, <i>Erethizon dorsatum</i> L.	239
HIGHBY, PAUL R. Mosquito vectors and larval development of <i>Dipetalonema arbuta</i> Highby (Nematoda) from the porcupine, <i>Erethizon dorsatum</i>	243
HIGHBY, PAUL R. Vectors, transmission, development, and incidence of <i>Dirofilaria scapiceps</i> (Leidy, 1886) (Nematoda) from the snowshoe hare in Minnesota	253
Hippoboscidae from southern Brasil	131
Histamine, estimation of, in rats and guinea pigs infected with <i>Trichinella spiralis</i>	367
<i>Histomonas meleagridis</i> , new medium for cultivation of	353
Hoffman, William Albert (1894-1943), portrait and in memoriam	301
Host, maintenance of a trematode, <i>Aspidogaster conchicola</i> , outside body of natural	127
Host records, new, of nematodes from Mustelidae (Carnivora)	158
Host-parasite relations between laboratory mice and <i>Nematospiroides dubius</i> Baylis	303
Hosts, fish, of <i>Posthodiplostomum minimum</i> , experimental studies on	350
HUNNINEN, A. V. AND R. M. CABLE. The life history of <i>Lecithaster confusus</i> Odhner (Trematoda: Hemiuridae)	71
HUNTER, GEORGE W., III. Specimens needed by the medical schools	160
HURLBUT, HERBERT S. Observations on the use of sea water in the control of <i>Anopheles albimanus</i> Wied.	356
HURLBUT, HERBERT S. The rate of growth of <i>Anopheles quadrimaculatus</i> in relation to temperature	107
<i>Hymenolepis nana</i> var. <i>fraterna</i> , dwarf tapeworm, development of, from mice of two different localities	423
<i>Hymenolepis nana</i> var. <i>fraterna</i> , increased infectivity of eggs of, following storage in host feces	417
<i>Hymenolepis nana</i> var. <i>fraterna</i> , relationship between intestinal size of young mice and their susceptibility to infection with	61
Immunity against <i>Trichinella spiralis</i> , natural transmission of, from mother rats to offspring	114
Immunity in rats against <i>Trichinella spiralis</i> , number of larvae and time required to produce	123
Incidence of blood parasites in birds from Georgia	153
IN MEMORIAM	
Albert Hassall (1862-1942)	233
William Albert Hoffman (1894-1943)	301
Winfield Carey Sweet (1891-1942)	365
Iowa, northwest, larval trematodes of	330; 340; 347
JACHOWSKI, LEO, JR. The oriental rat flea (<i>Xenopsylla cheopis</i>) in Michigan	300
JONES, ARTHUR W. A further description of <i>Stempellia moniezi</i> Jones, 1942, a microsporidian parasite (Nosematidae) of cestodes	373
JONES, HOWARD A. (see Jones and Jones)	151
JONES, WILLIAM R. AND HOWARD A. JONES. Preliminary observations on the efficacy of a product from oil of rose geranium for the removal of intestinal parasites from dogs	151
JORDAN, HELEN B. Blood protozoa of birds trapped at Athens, Georgia	260
JOURNAL OF PARASITOLOGY:	
New Editorial Committee	444

Kansas, southeastern, parasites of fox squirrel, <i>Sciurus niger rufiventer</i> , in	159
KIRBY, HAROLD. Observations on a trichomonad from the intestine of man	422
KNIPLING, EDWARD F. (see Brody and Knipling)	59
KUNTZ, ROBERT E. Cysticercus of <i>Taenia taeniaeformis</i> with two strobilae	424
LARSH, JOHN E., JR. Comparing the percentage development of the dwarf tapeworm, <i>Hymenolepis nana</i> var. <i>fraterna</i> , obtained from mice of two different localities	423
LARSH, JOHN E., JR. Increased infectivity of the eggs of the dwarf tapeworm (<i>Hymenolepis nana</i> var. <i>fraterna</i>) following storage in host feces	417
LARSH, JOHN E., JR. The relationship between intestinal size of young mice and their susceptibility to infection with the cestode, <i>Hymenolepis nana</i> var. <i>fraterna</i>	61
Larvae and time required to produce immunity against <i>Trichinella spiralis</i> in rats	123
Larvae, anopheline mosquito, tray for collecting	229
Larvae of <i>Cochliomyia americana</i> C. and P., can they mature in carcasses?	59
Larval stages of <i>Plagiorchis muris</i> in first intermediate host, development of	81
Larval trematodes of northwest Iowa. I. Nine new xiphidiocercariae	330
Larval trematodes of northwest Iowa. II. Four new strigeids	340
Larval trematodes of northwest Iowa. III. A new collarless echinostome cercaria	347
<i>Lecithaster confusus</i> Odhner, life history of	71
Leech feeding on ligula	299
<i>Leishmania</i> and <i>Trypanosoma</i> , viability of, in cultures	275
Lenses of frogs, turtles, birds and mammals, development of eye flukes of fishes in	136
LEVINE, P. P. The selective action of sulfaguanidine on avian coccidia	362
Life cycle of <i>Australorbis glabratus</i> (Say, 1818) Pilsbry, 1934, snail intermediate host of <i>Schistosoma mansoni</i>	231
Life cycle of four intestinal coccidia of domestic rabbit	10
Life cycle of <i>Tamerlanea bragai</i> (Trematoda: Eucotylidae)	424
Life history of <i>Crepidostomum farionis</i> , trematode, in Colorado	379
Life history of <i>Lecithaster confusus</i> Odhner (Trematoda: Hemiuridae)	71
Ligula, leech feeding on	299
LINCICOME, DAVID RICHARD. Acanthocephala of the genus <i>Corynosoma</i> from the California sea-lion	102
LINDQUIST, WILLIAM D. (see Pratt and Lindquist)	176
<i>Lipoptenae depressa</i> Say, louse fly, a new bacterium associated with	80
Lungs of white-tailed deer, adults of <i>Protostrongylus coburni</i> in	158
Lungworms (<i>Protostrongylus</i>), of varying hares in eastern North America	397
LUTTERMOSER, GEORGE W. A note on the life cycle of <i>Australorbis glabratus</i> (Say, 1818) Pilsbry, 1934, a snail intermediate host of <i>Schistosoma mansoni</i>	231
Maintenance of trematode, <i>Aspidogaster conchicola</i> , outside body of its natural host	127
MALDONADO, JOSÉ F. A note on the life cycle of <i>Tamerlanea bragai</i> (Trematoda: Eucotylidae)	424
Man, observations on a trichomonad from the intestine of	422
MANTER, HAROLD W. One species of trematode, <i>Neoreniifer grandispinus</i> (Caballero, 1938), attached by another, <i>Mesocercaria marcianae</i> (LaRue, 1917)	387
Medical schools, specimens needed by	160
MELENEY, HENRY E. The rôle of parasitologists in World War II	1
MELENEY, HENRY E. (see Snyder and Melenev)	278
MESERVE, F. G. <i>Phyllodistomum coatneyi</i> n. sp., a trematode from the urinary bladder of <i>Ambystoma maculatum</i> (Shaw)	226
<i>Mesocercaria marcianae</i> (LaRue, 1917), a trematode attacking another trematode (<i>Neoreniifer grandispinus</i>)	387
<i>Mesocercoides variabilis</i> Mueller, 1928, from opossum, observations on segmental anatomy of	217
Metacercariae, cultivation in vitro, free from microorganisms	319
Mice and <i>Nematospiroides dubius</i> , host-parasite relations between	303
Mice from different localities, development of dwarf tapeworm obtained from	423
Mice, intestinal size and susceptibility to infection with <i>H. nana fraterna</i>	61
Michigan, northern, two new large-tailed cercariae from	182
Michigan, oriental rat flea (<i>Xenopsylla cheopis</i>) in	300
Michigan, southern, parasites of mink in	361
Mink in southern Michigan, parasites of	361
Minnesota, vectors, transmission, development and incidence of <i>Dirofilaria scapiceps</i> from snowshoe hare in	253

MOHAN, BADRI NATH (see Russell, Mohan and Putnam)	208
MORGAN, BANNER BILL. New host records of nematodes from Mustelidae (Carnivora)	158
Mosquitoes, flagellate parasites of, with special reference to <i>Crithidia fasciculata</i>	196
Mosquitoes, flowers as suggested source of, and mosquito records in Dakota in 1941	328
Mustelidae (Carnivora), new host records of nematodes from	158
Myiasis, human intestinal caused by <i>Eristalis</i> , additional records of	425
NELSON, E. CLIFFORD. Cultivation of <i>Nyctotherus cordiformis</i>	292
Nematode (<i>Porrocaecum brevispiculum</i>), from the robin	161
Nematodes from Mustelidae (Carnivora), new host records of	158
<i>Nematospiroides dubius</i> Baylis and mice, host-parasite relations between	303
<i>Ncorenifer grandispinus</i> (Caballero, 1938), trematode, attacked by another (<i>Mesocercaria marcianae</i>)	387
New genera (indicated *) and new species (volume 29, 1943) :	
<i>Cambarincola macrocephela</i> Goodnight	100
<i>Cercaris aalbui</i> Brooks	337
<i>Cercaria ameeli</i> Hedrick	182
<i>Cercaria argenti</i> Brooks	335
<i>Cercaria conniae</i> Brooks	334
<i>Cercaria diamondi</i> Brooks	333
<i>Cercaria dorotti</i> Brooks	336
<i>Cercaria kingi</i> Brooks	331
<i>Cercaria lajeae</i> Brooks	330
<i>Cercaria leplei</i> Brooks	341
<i>Cercaria limosae</i> Hedrick	183
<i>Cercaria nolfi</i> Brooks	337
<i>Cercaria okobojensis</i> Brooks	343
<i>Cercaria ornatocauda</i> Brooks	348
<i>Cercaria pili</i> Brooks	334
<i>Cercaria stephensi</i> Brooks	340
<i>Cercaria stonii</i> Brooks	341
<i>Corynebacterium lipoptenae</i> Steinhaus	80
<i>Corynosoma obtusens</i> Lincicome	103
<i>Costia pyriformis</i> H. S. Davis	385
* <i>Dictyangium chelydrae</i> Stunkard	144
<i>Dipetalonema arbuta</i> Highby	239
* <i>Ewingella americana</i> Vail and Augustson	421
<i>Lynchia plaumanni</i> Bequaert	132
<i>Opecoelina pharynmagna</i> Annereaux	155
<i>Phyllodistomum coatneyi</i> Meserve	226
<i>Porrocaecum brevispiculum</i> Webster	162
* <i>Protechinostoma mucronisertulatum</i> Beaver	65
* <i>Spirogregarina fusiformis</i> Wood and Herman	193
<i>Strongyloides planiceps</i> Rogers	160
<i>Xironodrilus appalachi</i> Goodnight	100
New York, <i>Dispharynx spiralis</i> in golden and ring-necked pheasants in	230
<i>Nippostrongylus muris</i> infection in albino rats, characteristics of the population available for bioassay of anthelmintics in	42
Nodular worm larvae, overwinter loss of, from sheep pasture, and its bearing on control of nodular worm disease	263
North America, eastern, lungworms of varying hares in	397
<i>Nyctotherus cordiformis</i> , cultivation of	292
Ohio institution for children, pinworm infection in	298
OLIVIER, LOUIS (see Cort and Olivier)	81; 164
Opossum, observations on segmental anatomy of tapeworm <i>Mesocestoides variabilis</i> Mueller, 1928, from the	217
<i>Ornithodoros hermsi</i> Wheeler, Herms and Meyer, contribution to the biology of	33
<i>Ornithodoros nicolleti</i> Mooser, argasid tick, studies on biology of	393
Overwinter loss of nodular worm larvae from sheep pasture, and its bearing on control of nodular worm disease	263
Oyster-drilling snail (<i>Thais floridana haysae</i>), two flatworms from	362

PACKCHANIAN, A. On the viability of various species of <i>Trypanosoma</i> and <i>Leishmania</i> cultures	275
Parasite, microsporidian (Nosematidae), of cestodes, a further description of <i>Stempellia moniesi</i> Jones, 1942	373
Parasites, blood, in birds from southwestern United States	187
Parasites, blood, incidence of, in birds from Georgia	153
Parasites, intestinal, efficacy of product from oil of rose geranium for removal of, from dogs	151
Parasites of fox squirrel (<i>Sciurus niger rufiventer</i>) in southeastern Kansas	159
Parasites of mink in southern Michigan	361
Parasites of mosquitoes	196
Parasitization, modification of digestive gland tubules in snail <i>Stagnicola</i> , following	176
Parasitologists, rôle of, in World War II	1
<i>Pasteurella tularensis</i> , further attempts to transmit, by the bedbug	395
PETERS, HAROLD T. Studies on the biology of <i>Anopheles walkeri</i> Theobald (Diptera: Culicidae)	117
Pheasants, golden and ring-necked, <i>Dispharynx spiralis</i> in, in New York	230
PHILIP, CORNELIUS B. Flowers as a suggested source of mosquitoes during encephalitis studies, and incidental mosquito records in the Dakotas in 1941	328
<i>Phyllodistomum etheostomae</i> Fischthal, 1942 (Trematoda: Gorgoderidae), from percid fishes	7
Physalopteran (Nematoda) from domestic pig	229
Pig, domestic, a physalopteran (Nematoda) from	229
Pinworm infection in Ohio institution for children	298
<i>Plagiorchis muris</i> Tanabe, 1922, development of larval stages of, in first intermediate host ..	81
<i>Plasmodium gallinaceum</i> studies, spleen volume in domestic fowls in	208
<i>Polymorphus obtusus</i> Van Cleave, 1918 (Acanthocephala), a redescription of	289
Porcupine (<i>Erethizon dorsatum</i>), <i>Dipetalonema arbuta</i> n. sp. from	239
Porcupine, mosquito vectors and larval development of <i>Dipetalonema arbuta</i> Highby, from the	243
Portrait	
Hassall, Albert	232
Hoffman, William Albert	301
Meleney, Henry E. Frontispiece	
Sweet, Winfield Carey	364
<i>Posthodiplostomum minimum</i> , experimental studies on the fish hosts of	350
PRATT, IVAN AND WILLIAM D. LINDQUIST. The modification of the digestive gland tubules in the snail <i>Stagnicola</i> following parasitization	176
PRICE, E. W. AND G. DIKMANS. In memoriam. Albert Hassall (1862-1942)	233
<i>Protechinostoma mucronisertulatum</i> n. g., n. n. (<i>Psilostomum reflexae</i> Feldman, 1941), a trematode from the sora rail, studies on	65
<i>Protostrongylus coburni</i> , notes on adults of, in lungs of white-tailed deer	158
<i>Protostrongylus</i> lungworms of varying hares (<i>Lepus americanus</i>), in eastern North America	397
Protozoa of blood of birds trapped at Athens, Georgia	260
Psilostomidae, two new large-tailed cercariae from northern Michigan	182
PUTNAM, PERSIS (see Russell, Mohan and Putnam)	208
Quail, food habits and intensity of coccidian infection in	206
Rabbit, domestic, life cycle of four intestinal coccidia of	10
Rabbits, domestic, a new ectoparasite (Acarina: Cheyletidae) from	419
Rail, sora, studies on <i>Protechinostoma mucronisertulatum</i> from	65
Rat, kangaroo, <i>Giardia</i> in blood of	423
Rats, active immunity in, against <i>Trichinella spiralis</i>	123
Rats, albino, bioassay of anthelmintics in	42
Rats, infected with <i>T. spiralis</i> , estimation of histamine in	367
Rats, natural transmission of immunity against <i>Trichinella spiralis</i> from mothers to offspring	114
Redescription of <i>Contracaecum habena</i> (Linton, 1900) Linton, 1934	156
Redescription of <i>Polymorphus obtusus</i> Van Cleave, 1918 (Acanthocephala)	289
REID, W. M. A physalopteran (Nematoda) from the domestic pig	229
Robin, helminths from, with description of new nematode, <i>Porrocaecum brevispiculum</i>	161
Rodent warble flies (Cuterebridae)	311

ROGERS, WILLIAM P. <i>Strongyloides planiceps</i> , new name for <i>S. cati</i> Rogers	160
ROZEBOOM, L. E. In memoriam. William Albert Hoffman (1894-1943)	301
RUSSELL, PAUL F., BADRI NATH MOHAN AND PERSIS PUTNAM. Some observations on spleen volume in domestic fowls in the course of <i>Plasmodium gallinaceum</i> studies	208
RUTHERFORD, ROBERT L. The life cycle of four intestinal coccidia of the domestic rabbit ...	10
SAARNI, ROY W. (see Herman, Chatten and Saarni)	206
<i>Sarcocystis rileyi</i> (Stiles, 1893) in domestic fowl, <i>Gallus gallus</i>	300
SARLES, MERRITT P. Overwinter loss of nodular worm larvae from a sheep pasture and its bearing on the control of nodular worm disease	263
SCHECHTER, VICTOR. Two flatworms from the oyster-drilling snail, <i>Thais floridana haysae</i> Clench	362
<i>Schistosoma mansoni</i> , a note on the life cycle of <i>Australorbis glabratus</i> (Say, 1918) Pilsbry, 1934, a snail intermediate host of	231
Schistosome, <i>Cercaria stagnicola</i> Talbot, 1936, development of sporocysts of	164
<i>Sciurus niger rufiventer</i> , fox squirrel, in southeastern Kansas, parasites of	159
SEALANDER, JOHN A. Notes on some parasites of the mink in southern Michigan	361
Sea-lion, California, <i>Acanthocephala</i> of genus <i>Corynosoma</i> from	102
Sheep host, wandering of <i>Haemonchus</i> in	407
Sheep pasture, overwinter loss of nodular worm larvae from	263
SHORB, D. A. Survival on grass plots of eggs and preinfective larvae of the common sheep stomach worm, <i>Haemonchus contortus</i>	284
Snail (<i>Australorbis glabratus</i>), intermediate host of <i>Schistosoma mansoni</i> , note on life cycle of	231
Snail (<i>Thais floridana haysae</i>), two flatworms from	362
Snail (<i>Stagnicola</i>), modification of digestive gland tubules in, following parasitization ...	176
SNYDER, THOMAS C. AND HENRY E. MELENEY. Anaerobiosis and cholesterol as growth requirements of <i>Endamoeba histolytica</i>	278
Spleen volume in domestic fowls in <i>Plasmodium gallinaceum</i> studies	208
Sporocysts of schistosome, <i>Cercaria stagnicola</i> Talbot, 1936, development of	164
SPURLOCK, G. M. Observations on host-parasite relations between laboratory mice and <i>Nematospiroides dubius</i> Baylis	303
Squirrel (<i>Sciurus niger rufiventer</i>), in southeastern Kansas, parasites of	159
<i>Stagnicola</i> , snail, modification of digestive gland tubules in, following parasitization	176
STEINHILF, EDWARD A. A new bacterium, <i>Corynebacterium lipoptenae</i> , associated with the louse fly, <i>Lipoptena depressa</i> Say	80
<i>Stempellia moniezi</i> Jones, 1942, microsporidian parasite (Nosematidae) of cestodes, further description of	373
STOLL, NORMAN R. The wandering of <i>Haemonchus</i> in the sheep host	407
Strigeids, larval, four new, from northwest Iowa	340
<i>Strongyloides planiceps</i> , new name for <i>S. cati</i> Rogers	160
Strongyloidiasis, canine, case of, in Texas	157
STUNKARD, HORACE W. A new trematode, <i>Dictyogonium chelydrae</i> (Microscaphidiidae = Angiodictyidae), from the snapping turtle, <i>Chelydra serpentina</i>	143
Sulfaguanidine, selective action of, on avian coccidia	362
Survival on grass plots of <i>Haemonchus contortus</i>	284
Susceptibility to infection of young mice with <i>H. nana fraterna</i>	61
Sweet, Winfield Carey (1891-1942), portrait and in memoriam	364
<i>Taenia taeniaeformis</i> , cysticercus with two strobilae	424
<i>Tamerlanea bragai</i> , note on life cycle of	424
Technique, bioassay, for anthelmintics	48
Temperature, rate of growth of <i>Anopheles quadrimaculatus</i> in relation to	107
Texas, case of canine strongyloidiasis in	157
<i>Thais floridana haysae</i> Clench, oyster-drilling snail, two flatworms from	362
<i>Thelastoma icemi</i> (Schwenk), a nematode from cockroaches	404
THOMAS, H. DUKE. Preliminary studies on the physiology of <i>Aedes aegypti</i> (Diptera: Culicidae). I. The hatching of the eggs under sterile conditions	324
THOMPSON, PAUL E. The relative incidence of blood parasites in some birds from Georgia ..	153
Tick (<i>Ornithodoros nicolleti</i>), studies on biology of	393
TODD, A. C. <i>Thelastoma icemi</i> (Schwenk), a nematode from cockroaches	404
Trematode (<i>Neoreenifer grandispinus</i>), attacked by another (<i>Mesocercaria marcianae</i>)	387
<i>Trichinella spiralis</i> , estimation of histamine in rats and guinea pigs infected with	367

<i>Trichinella spiralis</i> , natural transmission of immunity against, from mother rats to offspring ..	114
<i>Trichinella spiralis</i> , number of larvae and time required to produce active immunity in rats against	123
Trichomonad from intestine of man, observations on a	422
Trout, a new polymastigine flagellate (<i>Costia pyriiformis</i>) parasitic on	385
<i>Trypanosoma</i> and <i>Leishmania</i> , viability of, in cultures	275
<i>Trypanosoma vespertilionis</i> (= <i>T. cruzi</i> ?), new locality for, in bats in United States	363
Turtle (<i>Chelydra serpentina</i>), a new trematode, <i>Dictyangium chelydrae</i> , from	143
UHRICH, JACOB (see Graham and Uhrich)	159
United States, new locality for <i>Trypanosoma vespertilionis</i> (= <i>T. cruzi</i> ?) in bats in	363
United States, southwestern, blood parasites in birds from	187
VAIL, EDWARD L. AND G. F. AUGUSTSON. A new ectoparasite (Acarina: Cheyletidae) from domestic rabbits	419
VAN CLEAVE, HARLEY J. AND CHARLES O. WILLIAMS. Maintenance of a trematode, <i>Aspidogaster conchicola</i> , outside the body of its natural host	127
Vectors and larval development of <i>Dipetalonema arbuta</i> Highby from the porcupine, <i>Erethizon dorsatum</i>	243
Vectors, transmission, development and incidence of <i>Dirofilaria scapiceps</i> (Leidy, 1886), from snowshoe hare in Minnesota	253
Viability of <i>Trypanosoma</i> and <i>Leishmania</i> in cultures	275
WALLACE, F. G. Flagellate parasites of mosquitoes with special reference to <i>Crithidia fasciculata</i> Léger, 1902	196
Wandering of <i>Haemonchus</i> in the sheep host	407
Warble flies (Cuterebridae), of rodents	311
WARD, HELEN L. A redescription of <i>Polymorphus obtusus</i> Van Cleave, 1918 (Acanthocephala)	289
WARD, JAMES W. (see Byrd and Ward)	217; 270
WEBSTER, J. DAN. Helminths from the robin, with the description of a new nematode, <i>Porrocaecum brevispiculum</i>	161
WEBSTER, J. DAN. The type of <i>Gyrocoelia milligani</i> Linton, 1927	230
WHEELER, CHARLES M. A contribution to the biology of <i>Ornithodoros hermsi</i> Wheeler, Herms and Meyer	33
WHITLOCK, J. H. Characteristics of the population available for bioassay of anthelmintics in <i>Nippostrongylus muris</i> infection in albino rats	42
WHITLOCK, J. H. AND C. I. BLISS. A bioassay technique for anthelmintics	48
WILLIAMS, CHARLES O. (see Van Cleave and Williams)	127
WOOD, SHERWIN F. AND CARLTON M. HERMAN. The occurrence of blood parasites in birds from southwestern United States	187
WOOD, SHERWIN F. A new locality for <i>Trypanosoma vespertilionis</i> (= <i>T. cruzi</i> ?) in bats in the United States	363
World War II, rôle of parasitologists in	1
<i>Xenopsylla cheopis</i> , oriental rat flea, in Michigan	300
<i>Xiphidiocercariae</i> , nine new, from northwest Iowa	330

JOURNAL OF PARASITOLOGY

New Editorial Committee

Beginning with the first number of Volume 30 (February 1944) the JOURNAL OF PARASITOLOGY will be in charge of the new Editorial Committee, which was appointed by Council of the American Society of Parasitologists on January 9, 1943 (J. Parasitol. 29: 238) for a 5-year period:

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